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Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.01/A1

Topic: A.02. Neurogenesis and Gliogenesis

Support: NEI R01EY021482

Title: The role of miR-124-9-9* in Müller glia reprogramming

Authors: *S. G. WOHL, K. E. COX, J. POLLAK, A. LATORRE, T. A. REH;
Dep. of Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Unlike lower vertebrates, the mammalian retina does not have the potential to regenerate after damage. Therefore, there is an obvious need for the development of new strategies to promote self-repair of the mammalian retina. We previously showed that lentiviral gene transfer of the transcription factor Achaete-scute homolog 1 (Ascl1) in murine Müller glia cultures resulted in partial reprogramming of the cells to retinal progenitors (Pollak et al., 2013). Recent evidence indicates that microRNAs (miRNAS) may also facilitate reprogramming of cells; for example, the brain enriched miRNA miR-124, can reprogram fibroblasts into functional neurons in combination with the miRNAs miR-9 and miR-9*. The aim of this study was to analyze whether 1) lentiviral gene transfer of miR-124-9-9* can reprogram Müller glia into retinal neurons and 2) miR-124-9-9* can improve Ascl1-induced reprogramming. Primary Müller glia cultures were generated from postnatal day (P) 12 mice, as shown previously (Ueki, 2012). Lentiviral particles, i.e. miR-124-9-9*-RFP, Ascl-1 GFP or GFP control, were added after one to two weeks in culture. Gene expression analyses or immunofluorescent stainings were performed within 2 weeks after infection. RT-qPCR data revealed that normal Müller glia cells expressed a variety of miRNAs, but brain enriched miR-124 was only expressed at low levels, consistent with our hypothesis. Overexpression of miR-124-9-9* via viral vectors showed that miR-124 levels were increased 16 fold on average, and this led to small increases in the neural progenitor genes, Atoh7, Lin28b, and Ascl1 when compared to GFP controls. When the viral vectors for Ascl1 and miR-124-9-9* were added together, RT-qPCR confirmed successful transduction for both constructs. Müller glial cultures infected with both viral vectors showed an increase in mRNA levels of Atoh7 as well as in the neuronal gene, Onecut2. Interestingly, three of the genes increased by miR-124-9-9* over-expression are associated with retinal ganglion cells (RGCs), a cell type normally generated early in retinal development. This suggests that the

inclusion of miR-124-9-9* in Müller glial reprogramming may facilitate the generation of early born retinal cell types, like ganglion cells.

Disclosures: **S.G. Wohl:** None. **K.E. Cox:** None. **J. Pollak:** None. **A. LaTorre:** None. **T.A. Reh:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.02/A2

Topic: A.08. Transplantation and Regeneration

Support: Hirschl/Weill-Caulier Research Award

Brain Research Foundation

NIH NINDS R21, NS088943

Title: Reprogramming MGE precursors to a glutamatergic fate

Authors: H. M. BELALCAZAR, *N. MCKEEHAN, J. M. HEBERT;
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Replacement of cortical projection neurons after injury or neurodegenerative diseases has been one of the main goals in the neuroregeneration field. Transplants of stem cells and cortical neural precursors have thus far failed as strategies for replacement due to the poor ability of these cells to disperse beyond the transplant site. In this study, we propose a different approach combining the ability of medial ganglionic precursor cells to disperse widely in the adult brain, and the reprogramming of these cells to a glutamatergic neuronal fate by transcription factors. A set of 15 transcription factors involved in glutamatergic neuron development is being evaluated *in vitro*. Neuronal precursors cells derived from medial ganglionic eminence of E12.5 mouse embryos, carrying the Rosa26;rtTA allele and Tau-mGFP-LacZ reporter, are plated and exposed to multiple combinations of bicistronic lentiviral vectors encoding transcription factors 5' to an ires-Cre. Doxycycline induction is maintained during 3 weeks. Cultures are fixed after 4 weeks and stained with beta galactosidase and Tbr1; the former is used as a transduction reporter and the later as a reprogramming readout. To date, no Tbr1+ cells as a result of reprogramming have been detected with a subset of tested combinations. Possible explanations could be negative interactions between transcription factors or a weak

expression of Tbr1 in reprogrammed cells. Further rounds of testing with additional transcription factors are being implemented as well as additional markers as a readout for reprogramming.

Disclosures: H.M. Belalcazar: None. **N. McKeehan:** None. **J.M. Hebert:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.03/A3

Topic: A.02. Neurogenesis and Gliogenesis

Support: CIRM Training Grant TG2-01165

NIH RO1

Title: Direct conversion of mouse and induced human fibroblasts to nicotine responsive neurons

Authors: *S. LEE, J. W. BLANCHARD, K. EADE, K. BALDWIN;
The Scripps.Research Insitute, San Diego, CA

Abstract: Nicotine addiction - despite the existence of a variety of smoking cessation therapies - remains one of the leading causes of death worldwide. Nicotine gains access to our nervous system via a class of cationic channels defined as nicotinic acetylcholine receptors (nAchRs). Different combinations of nine α (α2- α10) and three β (β2- β4) subunits are expressed by distinct sub-populations of neurons. In humans, genome-wide associations have linked predisposition to nicotine addiction to a cluster of single nucleotide polymorphisms at a genomic locus that includes three nAchR subunits (α3, β4 and α5). In the CNS, these subunits are highly expressed in the medial habenula (mH) and co-expression of nAChR α3 and α4 is nearly exclusive to a subset of mH neurons. Furthermore, mouse studies have linked these neurons and the expression of nAchR subunits in these neurons to the aversive and anxiolytic properties of nicotine. Being able to generate neurons expressing these nAchRs in a context that resembles their endogenous cell type would offer a new tool to probe molecular mechanisms governing nicotine responses. Additionally, it may provide a venue for identifying novel therapeutic compounds and establish the means by which human polymorphisms can influence propensity to nicotine addiction. Using a screening approach, we have identified a novel combination of virally transduced transcription factors can directly reprogram mouse embryonic fibroblasts (MEFs) into nicotine-responsive induced neurons that express the relevant nAChR α3, β4 and α5

genes. These factors also produce neurons from human fibroblasts. We will evaluate how closely these induced neurons resemble their endogenous counterparts in the mouse and establish how human genetic differences at the risk nAchR locus impact cellular responses to nicotine *in vitro*.

Disclosures: S. Lee: None. **J.W. Blanchard:** None. **K. Eade:** None. **K. Baldwin:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.04/A4

Topic: A.02. Neurogenesis and Gliogenesis

Support: Pritzker Neuropsychiatric Research Consortium

Title: Differentiation of SH-SY5Y human neuroblastoma cells alters FGF2-mediated signal transduction

Authors: *L. A. DOKAS, S. J. WATSON, H. AKIL;
Univ. of Michigan, Ann Arbor, MI

Abstract: Although it is clear that dysfunction of the fibroblast growth factor (FGF) system in the brain occurs in affective disorders, little is known regarding the signal transduction processes that mediate normal and pathological responses to FGF in neurons. The multiplicity of FGF ligands and FGF receptors on both neurons and glia complicate analysis of correlated cell signaling in the intact brain. For this reason, a relevant *in vitro* cell model is needed. The SH-SY5Y human (N-type) neuroblastoma cell line is useful in this regard as it expresses endogenous FGF receptors that, when activated, promote both proliferation and differentiation. In the undifferentiated state, FGF2 produces a rapid and sustained stimulation of extracellular stimulus-regulated kinase (ERK)1/2 phosphorylation. In contrast, there is a more modest and transient increase of Akt phosphorylation under the same conditions. Differentiation of SH-SY5Y cells according to the method of Lavenius et al. (1994) with 16 nM phorbol ester (12, 13-phorbol dibutyrate, PDB) and 3 nM FGF2 causes a mature neuronal phenotype characterized by outgrowth of processes with growth cone-like endings and elevated expression of the neuron-specific, growth-associated protein, GAP-43, and tyrosine hydroxylase. Also, differentiated cells show elevated ERK1/2 phosphorylation in comparison to control cells cultured for the same amount of time. However, this increase is reversed within 90 min. when differentiated cells are subsequently incubated in serum-free culture medium. Moreover, these cells no longer stimulate

ERK1/2 phosphorylation when re-exposed to FGF2. This change is specific to FGF2 as undifferentiated and differentiated cells increase ERK1/2 phosphorylation in response to either the cholinergic receptor agonist, carbachol (CCh), or to a lesser extent, insulin. After differentiation, FGF-mediated activation of Akt phosphorylation is also lost while the responses to CCh and, prominently to insulin, are maintained. These results demonstrate that although FGF2 promotes differentiation to a neuronal state, the response of differentiated cells to FGF2 is subject to modulation of the FGF receptor or post-receptor components of the ERK1/2 and/or Akt signal transduction pathways.

Disclosures: L.A. Dokas: None. **S.J. Watson:** None. **H. Akil:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.05/A5

Topic: A.02. Neurogenesis and Gliogenesis

Support: 2RO1 MH066912

Title: Generating targeted lhx6 human embryonic stem cell reporter lines for interneuron development research

Authors: *J. CHU[1], S. ANDERSON[1], A. KAYKAS[2];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Allen Inst. for Brain Res., Seattle, WA

Abstract: Cortical interneurons differentiated from human embryonic stem cell (hESC) provide a powerful tool to study interneuron development and interneuron dysfunction diseases. Isolation of putative cortical interneurons from a mixed population of differentiated hESCs by FACS requires faithful fluorescent markers. Lhx6, a homeodomain transcription factor, is expressed in most of the parvalbumin (PV) positive and somatostatin (SST) positive interneurons and their progenitors during development. We have generated Lhx6 reporter hESC lines by targeting Citrine fluorescent protein into the endogenous Lhx6 locus using transcription activator-like effector nucleases (TALENs). Lhx6-Citrine hESCs express Citrine after been differentiated into interneuron precursors and show colocalization of Citrine and endogenous Lhx6 protein. Citrine expression in Lhx6-Citrine cells is maintained after prolonged culture on cortical feeders. Lhx6-Citrine positive cells express GABAergic lineage markers such as GAD, vGAT and GABA. Further gene expression profiling of purified Lhx6-Citrine positive cells could reveal gene-

expression signature and cell surface markers for isolating cortical interneurons from unmodified human pluripotent stem cells.

Disclosures: J. Chu: None. **S. Anderson:** None. **A. Kaykas:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.06/A6

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Intramural

Title: Synaptamide ameliorates ethanol-induced impairment of neurogenic differentiation of neural stem cells

Authors: *M. RASHID, H.-Y. KIM;
LMS, NIAAA, NIH, Rockville, MD

Abstract: Prenatal exposure to ethanol is known to interfere with embryonic and fetal development and to cause abnormal neurodevelopment. Docosahexaenoic acid (DHA) is an omega-3 polyunsaturated fatty acid highly enriched in the brain that is considered essential for proper brain development and function. Recently, we found that *N*-docosahexenoyethanolamine (synaptamide), an endogenous metabolite of DHA, is a potent neurogenic factor for neural stem cell differentiation as low nM synaptamide significantly increased the number of MAP2 and Tuj-1 positive neurons with concomitant induction of PKA/CREB phosphorylation. In this study, we demonstrate that ethanol impairs differentiation of neural stem cells and synaptamide can reverse the ethanol effects at least in part. We found that chronic ethanol (25-100 mM) exposure by treating NSCs with ethanol-containing media daily for 4 days dose-dependently decreased the number of MAP2 and Tuj-1 positive neurons and PKA/CREB phosphorylation. We also found that cellular cAMP production was decreased dose-dependently by chronic ethanol treatment. Ethanol-induced cAMP reduction persisted in the presence of adenylate cyclase inhibitor (SQ22536) or non-selective and selective phosphodiesterase (PDE) inhibitors (caffeine, rollipram), indicating that ethanol acts on multiple targets. Ethanol significantly increased the cAMP-specific PDE4 level without affecting mRNA expression. Also, chronic ethanol reduced Gs alpha protein and mRNA expression, transiently, and decreased mRNA levels of adenylyl cyclase 7 (AC7) and AC8 as well as GTPγS binding. In contrast, synaptamide exerted opposite effects on cAMP production, PDE4 protein level and Gs alpha and AC expression. These results suggest that ethanol exposure impairs neuronal differentiation of NSCs while synaptamide

ameliorates the adverse impact of ethanol by counter-affecting shared targets in G-protein coupled receptor (GPCR) signaling including Gs alpha, AC and PDE4.

Disclosures: **M. Rashid:** None. **H. Kim:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

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Topic: A.08. Transplantation and Regeneration

Support: NIH GRANT NS049553-06

NIH GRANT NS041590-19

ALSA Q619D6

HARVARD STEM CELL INSTITUTE

Title: Induction of subtype-specific cortical projection neurons from endogenous cortical progenitors

Authors: A. OZKAN, *H. PADMANABHAN, S. L. SHIPMAN, J. D. MACKLIS; Harvard Univ., Cambridge, MA

Abstract: Neocortex is a relatively recently evolved and elaborated region of the forebrain of mammals that is responsible for higher cognitive, associative, and sensori-motor functions. Distinct subtypes of cortical projection neurons are selectively vulnerable in distinct neurodegenerative, developmental, and acquired diseases of the CNS. In particular, for this work, corticospinal motor neurons (CSMN) centrally degenerate (along with spinal motor neurons) in amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, and loss of motor function in spinal cord injury results from damage to CSMN axons in the corticospinal tract. Previous work has demonstrated that in constitutive neurogenesis in restricted regions of the adult brain, induction of limited neurogenesis, and transplantation of immature neurons, appropriate stage new, immature neurons are capable of integrating into adult circuitry, if synaptic space is available. *In situ* generation of specific subtypes of neurons from endogenous, partially fate-restricted progenitors offers a potential therapeutic approach for regeneration of diseased or injured cortical circuitry. Recent work by our lab and others has identified central

elements of nested, combinatorial "molecular logic" of stage- and state-specific transcription factor controls over development of broad classes and specific subtypes of cortical projection neurons. Here we apply selected, central molecular developmental controls toward directed differentiation of functional cortical projection neurons, with specified subtype from partially fate-restricted endogenous pallial (cortical) progenitors. The newly generated neurons exhibit appropriate morphological, molecular, and physiological properties compared to their primary mouse counterparts. Future work will assess the fidelity of their integration and function within complex cortical circuitry, both developmentally and in diseased brain.

Disclosures: A. Ozkan: None. **H. Padmanabhan:** None. **J.D. Macklis:** None. **S.L. Shipman:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.08/A8

Topic: A.02. Neurogenesis and Gliogenesis

Support: CIRM UCSD Stem Cell Training Grant II

NIH R01

Title: Novel transcription factor combinations induce distinct neuronal subtypes directly from fibroblasts

Authors: *R. K. TSUNEMOTO[1,2], J. W. BLANCHARD[1], K. T. EADE[1], P. A. CHUBUKOV[1], K. K. BALDWIN[1,2];
[1]The Scripps Res. Inst., La Jolla, CA; [2]Univ. of California San Diego, La Jolla, CA

Abstract: Direct reprogramming - the conversion of a non-neuronal cell type to a neuron with transcription factor overexpression - provides a unique tool to study the intrinsic regulation of neuronal subtype identity. Previously published studies have demonstrated that specific neuronal subtypes (excitatory, spinal motor, and dopaminergic neurons) can be directly reprogrammed from mouse and human fibroblasts *in vitro* by ectopically expressing different combinations of transcription factors. However, it is yet to be determined whether this technique can produce all or only some neuronal subtypes that resemble those found in the brain and how to best select for factors capable of programming subtype identity. To address these questions, we have enacted a

large transcription factor screen to discover novel combinations that generate induced neurons from mouse fibroblasts. We have, thus far, discovered a surprisingly large number of new transcription factor combinations that produce neuron-like cells (i.e. express Tuj1 and Map2). We will present additional data characterizing these putative induced neurons with respect to their electrophysiologic properties and patterns of gene expression. These studies will reveal common themes in direct reprogramming that will aide in future attempts to produce biomedically relevant neuronal subtypes.

Disclosures: R.K. Tsunemoto: None. **K.K. Baldwin:** None. **J.W. Blanchard:** None. **K.T. Eade:** None. **P.A. Chubukov:** None.

Poster

681. Induced Neurogenesis

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 681.09/A9

Topic: G.06. Computation, Modeling, and Simulation

Title: Differentiating human pluripotent stem cells to midbrain dopaminergic neurons

Authors: *S. SHIN[1,2], M. COKONIS[2], Y. YAN[2], K. VEDVIK[3], L. REICHILING[3], R. NEWMAN[2], R. FISHER[3], B. HANSON[3], M. VEMURI[2], D. KUNINGER[2];
[1]Primary and Stem cell Systems, LIFE Technologies, Primary and Stem Cell Systems, Frederick, MD; [2]Thermo Fisher Scientific, Frederick, MD; [3]Thermo Fisher Scientific, Madison, WI

Abstract: Specialized cell culture media is a foundational tool for researchers working in diverse areas, from basic and applied research to biopharmaceutical applications. Thermo Fisher Scientific offers media systems for culture of human and rodent (primary) neural cell types and more recently has focused on identifying conditions that drive stem cell differentiation toward specific neural lineages. AIM: To develop new cell culture systems that enable robust differentiation of human pluripotent stem cells (PSCs) to Midbrain Dopaminergic (mDA) Neurons. METHODS: We have adopted a multifaceted approach for driving PSC to neuronal differentiation- 1.Disconnecting specification/regionalization studies from maturation, allowing experimental time line compression and enabling parallel development activities. 2. Utilizing complex Design of Experiment (DOE) approaches and mathematical modeling paired with validated endpoint assays; 3. Incorporating small molecule chemical library screening to identify compounds with desired properties. RESULTS: We demonstrate the feasibility of distinguishing PSC specification from neuronal maturation by utilizing two different cell models of PSC and

NSC. The NSCs provide a good model to screen and optimize conditions driving neuronal differentiation and maturation. Additional results of definitive screening DOEs as well as modeling predictions are described. CONCLUSIONS: In the last several years significant advances in stem cell biology have enabled broader adoption of these cells and provided deeper insight into the mechanisms which regulate their growth and specific cell fate determination. In this work we present our approach to harness this insight to develop next generation culture systems to create authentic midbrain dopaminergic neurons from PSCs.

Disclosures: **S. Shin:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **M. Cokonis:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **Y. Yan:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **K. Vedvik:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **L. Reichiling:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **R. Newman:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **R. Fisher:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **B. Hanson:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **M. Vemuri:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **D. Kuninger:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.01/A10

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: BBSRC SA5818

Title: Measurement of acetylcholine release in the hippocampus during behavior using amperometric sensors

Authors: *L. M. TELES-GRILO RUIVO[1], K. L. BAKER[2], K. G. PHILLIPS[3], M. W. CONWAY[3], P. J. KINSLEY[3], J. R. HUXTER[3], G. GILMOUR[3], J. P. LOWRY[2], J. T. ISAAC[3], J. R. MELLOR[1];
[1]Sch. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom; [2]Dept. of Chem., Natl. Univ. of Ireland, Maynooth, Ireland; [3]Lilly Ctr. for Cognitive Neurosci., Eli Lilly and Co. Ltd., Windlesham, United Kingdom

Abstract: Acetylcholine plays a critical role in hippocampal function by controlling cellular, synaptic and network properties. However, we have limited information on the temporal and spatial profile of acetylcholine release during different behavioral states. Insight into the dynamics of acetylcholine release in the rodent brain has so far largely been provided by microdialysis studies, which have temporal resolution of the order of minutes and a spatial resolution >100μm. With the development of biosensors that use enzymatic redox reactions to detect the release of neurotransmitters it has become possible to measure neurotransmitter release at sub-second timescales and at spatial resolutions <100μm. For the measurement of acetylcholine, the enzyme choline oxidase is embedded into a polymer matrix coated on a metal electrode that detects the current produced by the oxidation of choline. Thus, these sensors detect choline production resulting from acetylcholine breakdown by endogenous acetylcholinesterase. Fast choline transients that increase and decrease over a period of a few seconds have been reported in the prefrontal cortex of rats performing an attentional task. However, the kinetics of acetylcholine release in the hippocampus of freely moving animals at this level of temporal resolution is largely unknown. Here we report changes in acetylcholine release in the dorsal hippocampus of adult mice during different behavioral states detected by video and intrahippocampal local field potentials. We first correlate these changes with different phases of sleep including REM, and non-REM as well as phases of wakefulness including physical activity and quiet resting. Finally, we correlate changes in acetylcholine release during performance of mice on a hippocampal-dependent behavioral task. We find that acetylcholine release is dynamically regulated during sleep and correlates with specific behavioral states.

Disclosures: **L.M. Teles-Grilo Ruivo:** None. **K.L. Baker:** None. **K.G. Phillips:** None. **M.W. Conway:** None. **P.J. Kinsley:** None. **J.R. Huxter:** None. **G. Gilmour:** None. **J.P. Lowry:** None. **J.T. Isaac:** None. **J.R. Mellor:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

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Program#/Poster#: 682.02/A11

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH R01 DC009836

NIH R01 DC009477

HHMI International Student Research Fellowship

Title: Elevated cholinergic tone disrupts the balance of stability and plasticity in the auditory cortex of ChAT-ChR2-EYFP transgenic mice

Authors: *W. GUO[1,2], B. G. SHINN-CUNNINGHAM[2], D. B. POLLEY[1,2,3];
[1]Eaton Peabody Lab., Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Ctr. for Computat. Neurosci. and Neural Technol., Boston Univ., Boston, MA; [3]Dept. Otology and Laryngology, Harvard Med. Sch., Boston, MA

Abstract: Acetylcholine (ACh) plays a critical role in regulating brain functions such as attention, learning, and memory. Whereas below-normal cholinergic tone in the cerebral cortex has been linked to memory deficits found in neurodegenerative disorders such as Alzheimer's disease, the physiological consequences of elevated cholinergic tone are less frequently discussed. Recently, a transgenic mouse line (ChAT-ChR2-EYFP) was developed to allow targeted optogenetic stimulation of cholinergic neurons. However, as an unintended side effect of the genetic manipulation, these mice overexpress the vesicular acetylcholine transporter gene (VAChT), leading to a constitutive elevation of ACh level throughout the brain. It has been shown that these mice suffer from severe cognitive deficits, especially in tasks involving attention and memory. In this study, we characterize the dysregulation of plasticity across cortical columns and topographic maps in the sensory cortex of these mice, which may speak to a generalized neural circuit dysfunction associated with hypercholinergia. During development, passive exposure to a repeating pure tone only modifies the receptive field organization of mouse primary auditory cortex (A1) during a brief critical period between P11-P15. We found that exposing adult ChAT-ChR2-EYFP mice to repeated 16 kHz tone pips (70 dB SPL, 250 ms at 2 Hz) rapidly modified the organization of the A1 tonotopic map and underlying receptive fields in contrast to both tone-exposed wild-type littermates and unexposed transgenic littermates. We also found that changes induced by tone exposure could be reversed within minutes upon presentation of a diverse set of tone stimuli. These data suggest that the floodgates for plasticity remain open in the cortex of adult ChAT-ChR2-EYFP mice, thereby permitting substantial, yet specific, remodeling of sensory representations in a manner reminiscent of critical period plasticity. The failure to transition from a labile state typical of early development to the stable sensory representations normally found in adult animals may contribute to the cognitive deficits recently described in ChAT-ChR2-EYFP mice. Our ongoing experiments are focused on delineating the laminar sequence of receptive field plasticity in awake mice, as well as mechanisms that regulate the induction and maintenance of these experience-dependent plasticity effects.

Disclosures: W. Guo: None. **B.G. Shinn-Cunningham:** None. **D.B. Polley:** None.

Poster

682. Brain Cholinergic Mechanisms

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Program#/Poster#: 682.03/A12

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Enterprise Ireland CFTD/2008/107

PRTLI (Cycle 4)

Title: The development of a microelectrochemical choline biosensor for real-time neurochemical monitoring

Authors: K. L. BAKER, F. B. BOLGER, *J. P. LOWRY;
Chem., Natl. Univ. of Ireland, Maynooth, Maynooth, Ireland

Abstract: Choline is the precursor and metabolite of the neurotransmitter acetylcholine. The availability of choline influences acetylcholine synthesis and release which is involved in learning and short term memory. The dysregulation of the cholinergic system is recognised as a determinant of cognitive decline in age-related neurodegeneration. Here we report the development of a biosensor for the detection of choline, which it is hoped will enable real-time in-vivo recording of this important component of the cholinergic system thus enabling targeted studies with the potential to offer a unique insight into various disease states. Long term in-vivo electrochemistry (LIVE) facilitates the direct sampling of the brain extracellular environment of freely moving animals through the implantation of microvoltammetric/amperometric sensors. LIVE involves the application of a potential across an electrode-solution interface to oxidise or reduce species close to the electrode surface in order to generate a faradaic current. This allows the change in concentration of a particular species to be measured continuously. The advantages of this technique include high temporal resolution, small probe size (reducing tissue damage) and minimal ECF chemical depletion. However, it is generally limited to the detection of electroactive species. Thus, for the detection of non-electroactive analytes (e.g. choline) in-vivo the development of a biosensor is required. The latter is a device that involves the immobilisation of a sensitive and selective biological element (usually an oxidase enzyme) on, or in close proximity of an analytical detector. Enzyme biosensors are the most thoroughly investigated sensors in the biosensor field. The amperometric enzyme electrode monitors the production of hydrogen peroxide from the reaction of the enzyme with its substrate. Utilising choline oxidase we have developed a novel choline biosensor which has been fully characterised in the in-vitro and in-vivo environments. This characterisation in-vitro, determined the sensitivity and selectivity of the sensor towards choline and potential endogenous interferents. The sensor was also characterised in the in-vivo environment and shown to successfully monitor neurochemical fluctuations in choline levels in behaving animals with excellent spatial and temporal resolution.

Disclosures: **K.L. Baker:** None. **F.B. Bolger:** None. **J.P. Lowry:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

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Program#/Poster#: 682.04/A13

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Wellcome Trust PhD studentship

Title: Modulation of hippocampal gamma oscillations by acetylcholine: An *in vitro* optogenetic and computational investigation

Authors: *R. BETTERTON[1], K. TSANEVA-ATANASOVA[2], J. MELLOR[1];
[1]Sch. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom; [2]Col. of Engineering, Mathematics and Physical Sci., Univ. of Exeter, Exeter, United Kingdom

Abstract: A neuronal oscillation can be broadly described as the rhythmic, synchronised firing of a population of cells. Gamma frequency oscillations (30-100 Hz) are associated with a variety of cognitive functions including attention, sensory processing and learning and memory. Acetylcholine (ACh) release is associated with an increase in the power of hippocampal gamma oscillations and selective knockout of various ACh receptor (AChR) subtypes has provided evidence to support this scenario. However, the detailed mechanism by which ACh regulates gamma oscillations remains unclear. To understand the mechanisms underlying the modulation of gamma oscillations by ACh we have utilised both *in vitro* and computational models. We developed an optogenetic system for eliciting gamma frequency oscillations in hippocampal slices. Male mice received stereotaxic injection into the CA3 region of the hippocampus of a viral vector (AAV5) containing channelrhodopsin (hChR2(H134R)) under the control of the CaMKIIα promoter. The resulting ChR expressing CA3 pyramidal cells elicited cellular and synaptic responses in response to 470 nm light stimulation. Stimulation of CA3 pyramidal cell bodies with short light pulses (5-50 ms) evoked action potentials and stimulation of Schaffer collateral axons elicited robust synaptic responses in the CA3 and CA1 regions that were blocked by the application of NBQX (10 μM) or TTX (1 μM). A 5 Hz sine wave of optical stimulation evoked robust theta-nested gamma oscillations with properties similar to those seen *in vivo*. In parallel, we developed a biophysical, Hodgkin-Huxley type computational model of an interconnected network of pyramidal cells and interneurons that produced activity within the gamma range, dependent on connectivity and input current. Consistent with the *in vitro* model, stimulation with a 5 Hz sine wave input to pyramidal cells induced theta-nested gamma

oscillations. Further model simulations predicted likely nodes of the network responsible for modulation of gamma oscillations. In both *in vitro* and computational systems, activation of AChRs increased the power of theta-nested gamma frequency oscillations confirming previous observations. In particular, the activation of M1 muscarinic receptors replicated most of the observations implicating M1 receptors as a major regulator of gamma frequency oscillations in the hippocampus.

Disclosures: R. Betterton: None. **K. Tsaneva-Atanasova:** None. **J. Mellor:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.05/A14

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIGMS 1K12GM102778

NS22061

DP1 NS082100

Title: Modulation of prefrontal cortical circuits by cholinergic inputs from the nucleus basalis

Authors: *G. Y. LOPEZ, D. A. TALMAGE, L. W. ROLE;
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Abstract: Understanding the mechanisms underlying the modulatory actions of acetylcholine (ACh) in the brain is essential to develop better therapeutic approaches that selectively control cholinergic tone. Our studies combine electrophysiological and optogenetic approaches to examine the contribution of the modulatory effects of ACh on synaptic transmission in the prefrontal cortex (PFC). At least 14 days prior to electrophysiological recording, floxed AAV1-oChIEF-dsTomato was stereotaxically delivered into the nucleus basalis of Meynert (NBM) of ChAT-Cre mice (post-natal day > 30). Red-labeled fibers in the PFC correspond to cholinergic projections from the NBM that are light activable. Our confocal microscopy studies reveal that the majority of pyramidal cells in PFC layer V are in close proximity with cholinergic fibers, in all three areas of the PFC (anterior cingulate, prelimbic, and infralimbic areas). Whole-cell patch clamp recording in acute brain slice from adult ChAT-Cre mice and optogenetic stimulation were then combined in order to examine the effects of the selective activation of NBM

cholinergic input to PFC layer V. Non-patterned 10 Hz opto stimulation of red-labeled fibers in the PFC resulted in a decrease in the spontaneous postsynaptic currents in layer V pyramidal neurons. These data, as well as preliminary studies of miniature excitatory and inhibitory postsynaptic currents, suggest that there is an overall increase in excitability of layer V pyramidal neuron that might be explained by a change in the excitatory/inhibitory (E/I) balance, due to ACh release in the PFC. The effects of ACh on the E/I balance in PFC layer V are consistent with idea that ACh increases activity in attention related circuits.

Disclosures: G.Y. Lopez: None. **D.A. Talmage:** None. **L.W. Role:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

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Program#/Poster#: 682.06/A15

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR Grant MOP 89919

CIHR Grant MOP 126000

Title: Abnormal hippocampal activation in freely behaving mice deficient for vesicular acetylcholine transporter

Authors: *S. MOALLEM[1], M. PRADO[2], V. PRADO[2], S. LEUNG[3];
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Abstract: Acetylcholine has a fundamental role in cortical activation. The activation of the hippocampus, a cortex implicated in cognitive and sensorimotor functions, is characterized by an increase in power and frequency of oscillations in the theta (4-10 Hz) and gamma (30-100 Hz) frequency range. We studied hippocampal activation in mice with deficiency in cholinergic functionality due to heterozygous knockdown (KD^{Het}) of the vesicular acetylcholine transporter gene. We hypothesized that the mutant KD^{Het} mice, relative to wild type (WT) mice, will manifest abnormal theta and gamma oscillations during different behaviors, and in response to muscarinic cholinergic antagonist scopolamine hydrochloride (5 mg/kg i.p.). Hippocampal electroencephalogram (EEG) was recorded from electrodes placed at CA1 stratum radiatum in

behaving WT and mutant KD^{Het} mice. Mutant mice manifested a significantly higher theta peak frequency during walking (8.2 ± 0.17 Hz, n=5), as compared to WT mice (7.2 ± 0.25 Hz, n=6). Theta power during walking was weaker in mutant than WT mice. The peak theta frequency during awake immobility was also higher in mutant (7.0 ± 0.06 Hz, n=5) than WT (6.0 ± 0.34 Hz, n=5) mice. Injection of scopolamine abolished the immobility-associated theta oscillations in all WT mice, but a theta peak remained at 3.9 ± 0.45 Hz in the 5 mutant mice. The presence of a scopolamine-resistant theta rhythm during awake immobility in mutant KD^{Het} mice is the novel finding, since immobility-associated theta rhythm was found to be scopolamine (or atropine) sensitive in WT mice and other laboratory animals. The compensatory changes that activate a scopolamine-resistant, presumably non-cholinergic, theta rhythm during immobility in KD^{Het} mice remains to be identified.

Disclosures: **S. Moallem:** None. **M. Prado:** None. **V. Prado:** None. **S. Leung:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.07/A16

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR MOP 89825

Canada Research Chairs Program

Early Researcher Award from the Province of Ontario

Title: Mechanisms of sustained cholinergic excitation of prefrontal executive circuitry

Authors: *E. PROULX, M. TIAN, L. KANG, E. K. LAMBE;
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Abstract: The cholinergic modulation of the prefrontal cortex is essential to working memory and attention. In particular, acetylcholine exerts robust excitation of prefrontal layer 6 pyramidal neurons to achieve optimal performance on challenging attention tasks. These cells are ideally positioned to influence executive circuitry by virtue of their cortico-cortical and dense cortico-thalamic connectivity. Here, we examine the mechanisms of cholinergic excitation of prefrontal layer 6 pyramidal cells with whole-cell electrophysiology in the adult brain slice preparation combined with optogenetic and pharmacological manipulations. Under conditions of baseline

firing elicited with current injection, acetylcholine application or its endogenous release elicits a substantial and prolonged acceleration in action potential firing. This acetylcholine-elicited increase in spiking is mediated by contributions from both muscarinic and nicotinic receptors. Since the relevant muscarinic receptors are linked to the modulation of multiple potassium channels known to shape the action potential and regulate peak firing rates, we have investigated the consequences of manipulating potassium channels on the effects of acetylcholine in this population of prefrontal layer 6 neurons. We identified one family of potassium channels essential for maintaining a sustained response to acetylcholine in these neurons. Our results point to a trade-off between excitation and spiking fidelity. This compromise may ultimately have important ramifications for the regulation of excitability of prefrontal executive circuits.

Disclosures: **E. Proulx:** None. **M. Tian:** None. **L. Kang:** None. **E.K. Lambe:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.08/A17

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Kuwait University

CIHR

Title: Alzheimer's Disease-like cognitive and pathological dysfunction in a genetic mouse model of forebrain cholinergic deficiency

Authors: ***M. AL-ONAIZI**[1], B. KOLISNYK[2], M. PRADO[1,2,3], V. PRADO[1,2,3];
[1]Anat. and Cell Biol., Robarts Res. Inst., London, ON, Canada; [2]Neurosci., [3]Physiol. and Pharmacol., Western Univ., London, ON, Canada

Abstract: Acetylcholine (ACh) plays a crucial role in controlling a number of physiological processes in both the peripheral and central nervous system. In Alzheimer's disease (AD) there is pronounced degeneration in basal forebrain cholinergic neurons, increased number of amyloid plaques with aggregated proteins and decreased neurogenesis. The precise role of ACh in cognitive functioning and its relationship to pathological and functional hallmarks of AD is still poorly understood. We hypothesized that deficits of cholinergic tone in the hippocampus lead to disruption in cognitive processes, characterized by changes in the molecular functionality of the

hippocampus. To test this hypothesis we generated a mouse line with a forebrain specific deletion of the vesicular acetylcholine transporter (VAChT; $VAChT^{NK\text{-}Cre\text{-}flox/flox}$), a protein required for synaptic storage and release of ACh. To analyze cognitive ability, mice were tested in a novel hippocampal-dependent paired associate learning (PAL) touchscreen task, a human version of which is currently utilized to identify individuals at high risk for developing AD. Robust deficits were seen in their performance in the PAL task for VAChT-deficient mice compared to controls. Because neurogenesis has been implicated in cognitive performance, we investigated neurogenesis markers in the Subgranular Zone. We found that absence of cholinergic activity in the hippocampus led to vigorous alterations in adult neurogenesis, similar to what is observed in AD. Furthermore, to test the possibility that decreased cholinergic tone can affect protein aggregation and plaque formation we used thioflavin-S, a dye that binds specifically to aggregated proteins. Interestingly, protein aggregation was considerably increased in aged, but not young, $VAChT^{NK\text{-}Cre\text{-}flox/flox}$ mice compared to controls. To assess if cortical and hippocampal neurons targeted by cholinergic activity present alterations in functionality, we used silver staining, which detects membrane abnormalities in cells that will undergo degeneration. Robust levels of positive silver staining were observed in the cortex and the hippocampus of aged $VAChT^{NK\text{-}Cre\text{-}flox/flox}$ mice compared to controls. These results indicate that this novel mouse model provides insight on how the basal forebrain cholinergic system modulates the functionality of the hippocampus at the cellular level and in controlling plaque formation, both of which are hallmarks of AD.

Disclosures: **M. Al-Onaizi:** None. **M. Prado:** None. **V. Prado:** None. **B. Kolisnyk:** None.

Poster

682. Brain Cholinergic Mechanisms

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSF DBI; Grant: 1126118

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Whittier College Faculty Research Grant

Title: Chlorpyrifos exposure induces abnormalities in developing cholinergic neurons in Caenorhabditis elegans and Xenopus laevis

Authors: M. QUELHORST[1], H. SCHMIDT[2], O. MAC[2], D. BOURGAIZE[2], *E. A. FRADINGER[2], F. L. WATSON[1];
[1]Biol., Washington and Lee Univ., Lexington, VA; [2]Biol. Dept., Whittier Col., WHITTIER, CA

Abstract: In the United States, approximately 33 million pounds of organophosphate pesticides are used annually to protect agricultural crops and residences from insects. These pesticides function by inhibiting acetylcholinesterase (AChE), an enzyme that degrades acetylcholine at the cholinergic synapse. Using sublethal levels of the organophosphate pesticide chlorpyrifos, we assess its impact on the development of the cholinergic nervous system of transgenic Caenorhabditis elegans and Xenopus leavis embryos expressing green fluorescent protein (GFP) in the motor and sensory neurons, respectively, and evaluate the AChE inhibition kinetics *in vitro*. In *C. elegans*, exposure to chlorpyrifos at concentrations of 10 μM resulted in a decrease in the number and expression pattern of DA and DB cholinergic ganglia at the L1 stage. In X. laevis, we use immunocytochemistry to identify two populations of GFP-expressing sensory neurons, Rohon-Beard and dorsal root ganglion neurons, and use confocal microscopy to assess the effect of chlorpyrifos exposure on the number and position of GFP-expressing sensory neurons in the spinal column during neurogenesis. Preliminary data show exposure to sublethal levels chlorpyrifos leads to defects in spinal architecture. Given the widespread use of organophosphate pesticide, it is critical to understand their impact on the neuronal development of non-target species.

Disclosures: M. Quelhorst: None. **E.A. Fradinger:** None. **D. Bourgaize:** None. **F.L. Watson:** None. **O. Mac:** None. **H. Schmidt:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

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Program#/Poster#: 682.10/A19

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR CGS DRA (MKT)

CIHR Grant MOP 89825 (EKL)

Canada Research Chairs Program (EKL)

Title: Nicotinic acetylcholine receptors and what else? Relative modulation of layer VI neuronal activity by acetylcholine, dopamine, and norepinephrine

Authors: *M. K. TIAN, E. PROULX, M. PIVA, E. K. LAMBE;
Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The prefrontal cortex is central to attentional performance. In particular, layer VI neurons of prefrontal cortex are an important source of the corticothalamic output necessary for top-down control of attention. Neurons in layer VI are modulated by both excitatory and inhibitory inputs from numerous regions of the brain as well as local cortical sources. However, it is not well understood how the typical prefrontal layer VI pyramidal neuron responds to such modulation. Understanding which neurotransmitters exert powerful control over layer VI neuronal activity is essential to appreciate a key neurobiological mechanism regulating attentional performance. Here, we present evidence of excitatory responses to cholinergic, dopaminergic, and noradrenergic stimulation of layer VI neurons of medial prefrontal cortex. Using whole-cell recording in acute brain slices from adult mice, we measured electrophysiological responses elicited by application of acetylcholine, dopamine, and norepinephrine. While all three neurotransmitters depolarized layer VI pyramidal neurons, activation of nicotinic receptors by acetylcholine produced a significantly larger maximal depolarization, compared to activation of dopaminergic and noradrenergic receptors. The excitatory effects of dopamine are mirrored by specific D1 receptor agonists. In addition to changes to membrane potential, we find a significant increase in neuronal excitability following application of acetylcholine and dopamine, in contrast to a decrease in excitability following norepinephrine. Further investigation of the effects of additional neurotransmitters and of potential interactions between neurotransmitters are ongoing. We are particularly interested to identify neurotransmitters which can significantly enhance or diminish the effects of acetylcholine.

Disclosures: **M.K. Tian:** None. **E. Proulx:** None. **M. Piva:** None. **E.K. Lambe:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

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Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR MOP 89825 (EKL)

Canada Research Chairs Program (EKL)

Ontario Early Researcher Award (EKL)

Title: Prefrontal layer VI pyramidal neuron spine density: Dependence on apical dendrite sculpting by nicotinic acetylcholine receptors

Authors: *L. KANG[1,2], C. D. BAILEY[4], M. K. TIAN[1], E. K. LAMBE[1,3];
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Abstract: Layer VI pyramidal neurons of the medial prefrontal cortex play an important role in the 'top-down' control of attention. In most cortical regions, these neurons typically have "short" apical dendrites that terminate within the deeper cortical layers. Yet in adult prefrontal cortex, approximately half of these layer VI neurons are "long" with apical dendrites stretching across the cortical mantle to the pial surface. Here, we examine differences in the density and localization of excitatory inputs to these two subgroups of prefrontal layer VI neurons. Unexpectedly, the long cells have significantly greater dendritic spine density on both their apical and basal dendrites, compared to the short prefrontal layer VI neurons. Strikingly, we find that the spine density of the long neurons is significantly reduced by certain manipulations of nicotinic acetylcholine receptors. These developmental manipulations have been linked to attention deficits as well as to altered adult proportions of long and short prefrontal layer VI neurons. The reduction in spine density is specific to the long neurons and depends on the loss of the alpha5 nicotinic subunit encoded by *chrna5,* implicated in the normal developmental sculpting of prefrontal layer VI neurons. These results suggest that perturbations to alpha5-containing nicotinic acetylcholine receptors may increase the risk for attention deficits by altering the typical pattern by which layer VI pyramidal neurons integrate excitatory input across the superficial and deep layers of prefrontal cortex.

Disclosures: L. Kang: None. **C.D. Bailey:** None. **M.K. Tian:** None. **E.K. Lambe:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.12/A21

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Subgroups of neurones in the mouse spinal cord co-localised with enzymes synthesising GABA and acetylcholine

Authors: *J. GOTTS[1], I. J. EDWARDS[2], S. A. DEUCHARS[1], J. DEUCHARS[1];
[1]The Fac. of Biol. Sci., Univ. of Leeds, Leeds, United Kingdom; [2]Div. of Biosci., Univ. Col. London, London, United Kingdom

Abstract: Neuronal circuits in the spinal cord are involved in many important functions; including sensory, motor and autonomic control. Two of the major neurotransmitters operating within the spinal cord are GABA and acetylcholine. There is evidence that GABA and acetylcholine can be released from the same cells, such as starburst amacrine cells in the retina (Lee et al., 2010). However, in the spinal cord GABA and acetylcholine co-transmission has not been well established. To further develop understanding of the interrelationship, the present study focussed on examining the distribution of ChAT immunoreactivity and co-localisation with green fluorescent protein (GFP) in the cervical, thoracic and lumbar cord sections of transgenic adult mice expressing GFP under control of the GAD67 promoter (GAD67-GFP mice) (Tamamaki et al., 2003). GAD67-GFP mice (4-6 weeks, n = 3) were injected intraperitoneally with 0.1 ml of 1 % Fluorogold. After a period of 24-48 hours the mice were anaesthetised intraperitoneally with 60 mg/kg sodium pentobarbitone, and perfused transcardially with 4 % paraformaldehyde. The tissues were sectioned at 50μm on a vibrating microtome and the sections were processed utilising double labelling immunofluorescence for ChAT and GFP. Finally, the number of co-localised neurones per section and the degree of co-localisation as a percentage was determined from 10 sections from each animal for each studied spinal region. Although neurones immunoreactive for ChAT or GAD67-GFP were found throughout the spinal cord, co-localisation of ChAT and GFP was observed primarily in lamina X. Within this lamina 9.04% (121/1338) of ChAT and GAD neurones in the cervical sections were ChAT-GFP co-localised (with an average detection rate of 4.03 ± 0.29 (mean ± SEM) co-localised neurones per section), 8.14% (114/1400) of such neurones were co-localised in the thoracic sections (average detection rate 3.80 ± 0.42 per section), and 9.76% (134/1373) of such neurones were co-localised in the lumbar sections (average detection rate 4.47 ± 0.59 per section). The majority of co-localised neurones appeared to be positioned ventral and ventrolateral to the central canal (based on a reconstruction of the thoracic cord serial sections). These co-localised neurones seem to be neither motor nor preganglionic neurones, since they were negative for Fluorogold. Given that the projections and functions of these co-localised neurones in lamina X have not been previously reported, their involvement in neuronal circuits will likely become an area of interesting future investigation.

Disclosures: J. Gotts: None. **I.J. Edwards:** None. **S.A. Deuchars:** None. **J. Deuchars:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

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Program#/Poster#: 682.13/A22

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant 2RO1HD42215

Title: Characterization of the zebrafish interpeduncular nucleus

Authors: *A. SUBEDI[1], E. DUBOUÉ[2], M. HALPERN[1];
[1]Biology/Embryology, Johns Hopkins University/ Carnegie Inst. For Sci., Baltimore, MD;
[2]Embryology, Carnegie Institutuion for Sci., Baltimore,, MD

Abstract: The interpeduncular nucleus (IPN) is a highly conserved structure among vertebrates located in the midline of the midbrain. In zebrafish, the IPN receives major synaptic input from neurons of dorsal habenular nuclei (dHb), which are equivalent to the medial habenular nuclei of mammals. The habenulointerpeduncular pathway has been implicated in multiple functions including stress, fear, reward, and sleep. However, the role of the IPN in mediating behavior is not well understood. Moreover, little is known about the populations of IPN neurons. To probe the molecular identity of IPN neurons, we performed transcriptional profiling by RNA-seq. The IPN was located and microdissected from the adult zebrafish brain using the transgenic line *TgBAC(gng8:Eco.NfsB-2A-CAAX GFP)*^{c375} that robustly labels the axons of innervating habenular neurons with membrane-tagged GFP. We compared RNA sequences prepared from the isolated IPN with those from the remainder of the brain and identified several genes that are highly enriched throughout the IPN. Transcripts that localized to discrete IPN subregions were also identified. The *somatostatin 1.1* (*sst1.1*) gene is expressed throughout the IPN, whereas *somatostatin 3* (*sst3*). transcripts are confined to cells in the ventral IPN. We generated a Tg*(sst1.1:mCherry-caax)*^{c459} transgenic line and discovered that *sst1.1* also labels a subset of neurons in the dorsal habenula with more in the right nucleus than the left. The *sst1.1* neurons innervate a dorsally restricted portion of the ventral IPN, further supporting IPN regional specialization. We are attempting to manipulate the dHb *sst1.1* neuronal population by blocking neurotransmission in order to gain insight into the precise function of this IPN subregion.

Disclosures: **A. Subedi:** None. **E. Duboué:** None. **M. Halpern:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.14/A23

Topic: B.07. Synaptic Transmission

Support: DP1 NS 082100

Title: Cholinergic modulation of pyramidal Neurons in the ventral Subiculum

Authors: *S. WANG[1], D. TALMAGE[2], L. ROLE[3];
[2]Dept. of Pharmacol. Sci., [3]Dept. of Neurobio. & Behavior, [1]Program In Neuroscience, SUNY At Stony Brook, Stonybrook, NY

Abstract: The ventral Subiculm (vSub), which receives a massive cholinergic input from the medial septal nucleus and diagonal band (MS-DB) complex, is a major output area of the hippocampus which projects to an array of emotion-related cortical and subcortical areas. Previous studies show that altering the cholinergic input in the vSub can change the vSub projection to other areas and thus profoundly affect vSub involved behaviors such as fear learning, stress, and reward seeking. However, very little is known about the mechanism by which acetylcholine (ACh) regulates output neurons of the vSub. This study is to determine how exogenous and endogenous ACh regulates the neural circuits in the vSub and how it regulates pyramidal projection neurons (PYNs). Our initial studies examine the effect of cholinergic agonists and antagonists on regulating vSub circuit, using local delivery of drugs in acute mouse brain slice preparation, and patch-clamp recording to assess the firing properties and macroscopic current response in PYNs. ACh (1mM) typically elicits inward and outward currents mediated by both nicotinic and muscarinic ACh receptors (nAChRs & mAChRs). ACh (1-10uM) elicits transient frequency change in the mini excitatory postsynaptic currents (mEPSCs) in PYNs. These data are consistent with the idea that ACh modulates the excitability of PYNs via both presynaptic and postsynaptic mechanisms. To examine the role of endogenous ACh in the modulation of vSub circuit, we optogenetically labeled cholinergic cells within the medial septal nucleus and diagonal band (MS-DB) complex. Activating ChIEF in cholinergic terminal fields within the vSub alters the amplitude and/or frequency of spontaneous EPSCs. Cholinergic responses in vSub PYNs likely involve a variety of pre- and post- synaptic mechanism for cholinergic modulation.

Disclosures: S. Wang: None. **D. Talmage:** None. **L. Role:** None.

Poster

682. Brain Cholinergic Mechanisms

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Topic: B.07. Synaptic Transmission

Support: NIH Grant R03 DC 11361

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SVM CORP Grant LAV 3385

Title: Cholinergic modulation of functional thalamocortical topography

Authors: *C. C. LEE[1], Y. YANAGAWA[2], K. IMAIZUMI[1];
[1]Comparative Biomed. Sci., LSU Sch. of Vet. Med., Baton Rouge, LA; [2]Dept. of Genet. and Behavioral Neurosci., Gunma Univ. Grad. Sch. of Med., Gunma, Japan

Abstract: The principal nuclei of the thalamus convey sensory information from the periphery to the primary sensory areas of the cerebral cortex. These connections are organized in a highly topographic manner with limited divergence and convergence in each sensory modality. This organizational plan establishes the canonical sensory maps observed in the cortex. These maps are plastic with experience and learning, which enables adaptive cortical processing to fit the sensory environment. Although the roles of neuromodulatory systems, such as the cholinergic system, in this adaptive process have been well established, the neural substrates that establish these remapped parameters remain uncertain. Therefore, we sought to examine the cholinergic modulation of functional inputs from the thalamus to the cortex. We utilized *in vitro* slice preparations that maintain the intact connections from the principal sensory thalamus to primary sensory cortex. We then assessed the functional topography of projections using whole-cell patch clamp recorded responses of cortical neurons to laser-scanning photostimulation via uncaging of glutamate in the thalamus. In this manner, we mapped the functional thalamocortical inputs following bath-applied agonists to nicotinic acetylcholine receptors (nAhCRs). We correlated these results with the neuroanatomical organization of cortical nicotinic acetylcholine receptor subtypes (α7 and β2). We found that the thalamocortical projections form highly topographic functional connections in our slice preparation that expand in the presence of bath applied nAhCR agonists. In addition, the photostimulation-evoked EPSPs in the cortex also exhibited an increase in amplitude. This correlates with the neuroanatomical distribution of nAChR subtypes

in the cortex. These cholinergic modifications of functional thalamocortical circuitry may account for *in vivo* physiological properties and provide the neural substrates for learning of remapped sensory parameters.

Disclosures: C.C. Lee: None. **Y. Yanagawa:** None. **K. Imaizumi:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

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Topic: B.07. Synaptic Transmission

Support: NIH/NIEHS intramural research program

Title: Cholinergic regulation of the hippocampal area CA1 to the entorhinal cortex circuit

Authors: *J. HAAM, J. L. YAKEL;
NIH/NIEHS, Research Triangle Park, NC

Abstract: Memory formation consists of two stages, memory encoding and consolidation. During memory encoding, the neocortex transfers information to the hippocampus for the storage of temporary information. During memory consolidation, the temporary hippocampal information is transferred to the more permanent storage neocortex. The entorhinal cortex (EC) plays a key role in memory processing and consolidation by acting as a main interface between the hippocampus and neocortex. Hippocampal interneurons express high levels of nicotinic acetylcholine receptors (nAChR) and are highly sensitive to nicotine. We hypothesized that acetylcholine (ACh) regulates the switch between the two modes (encoding versus consolidation) by controlling hippocampal interneurons, which in turn controls the CA1-to-EC circuit. To study how ACh controls the circuit, we used electrophysiological recordings in septohippocampal co-cultured mouse brain slices. To examine the CA1-to-EC circuit, evoked excitatory postsynaptic currents (eEPSCs) were recorded in neurons in the EC layer V (ECV) with electrical stimulation of the CA1 pyramidal neurons. To selectively activate cholinergic neurons, we used optogenetics; channelrhodopsin ChR2 was selectively expressed in choline acetyltransferase (ChAT)-expressing neurons by injecting AAV9-dfloxed hChR2 to cultured slices from the transgenic ChAT-cre mice. Stimulation of cholinergic neurons by 488 nm light caused a significant decrease in eEPSC amplitude in ECV neurons, suggesting that ACh suppresses the CA1-to-EC pathway. The ACh-induced suppression of the CA1-to-EC circuit was

blocked by the α7 nAChR antagonist MLA. In addition, the ACh-induced suppression of the CA1-to-EC circuit was completely abolished by the $GABA_A$ receptor antagonist gabazine, implicating the role of GABAergic interneurons. This nAChR-induced inhibition of the CA1 to EC circuit provides a potential mechanism for acetylcholine regulation of memory encoding/consolidation, and helps us to better understand drugs that modulate acetylcholine actions and that may be useful in the treatment of Alzheimer's disease.

Disclosures: J. Haam: None. **J.L. Yakel:** None.

Poster

682. Brain Cholinergic Mechanisms

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Topic: B.07. Synaptic Transmission

Support: NSERC

FRQS

Title: The contribution of Ih to the cholinergic enhancement of repetitive synaptic responses at theta- and gamma-frequencies in the parasubiculo-entorhinal pathway

Authors: *D. W. SPARKS, C. A. CHAPMAN;
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Abstract: The entorhinal cortex provides the hippocampus with the majority of its sensory input, and also receives the major output projection from the parasubiculum. This puts the parasubiculum in a position to modulate sensory input to the hippocampus through its projection to the entorhinal cortex. Oscillatory activity in these brain areas at gamma- and theta-frequencies is modulated by cholinergic input from the medial septum, and these rhythms are thought to contribute to exploratory and mnemonic processes. We have previously found, using field recordings, that application of the cholinergic agonist carbachol (CCh) to slices of brain tissue resulted in a relative facilitation of short trains of stimulation delivered at both gamma (33Hz) and theta (10Hz) frequencies. This effect was found to depend on the non-specific cationic channel Ih. The purpose of the current study was to investigate the mechanisms behind the relative facilitation effect using intracellular recordings, which was accomplished through whole-cell patch clamp recordings in four to eight-week old male Long-Evans rats. A bipolar tungsten

stimulating electrode was placed in the parasubiculum, and a borosilicate glass recording electrode targeted neurons in layer II of the medial entorhinal cortex. The previous extracellular results were replicated, with CCh inducing a relative facilitation during train-evoked responses at both gamma and theta frequencies. This finding was accompanied by a reduction in inward-rectification during negative current injection, suggesting that this effect could be due to a reduction in Ih. Application of ZD7288, a potent Ih antagonist, mimicked the effects of CCh on train-evoked responses, supporting the idea that CCh acts through Ih to produce the relative facilitation effect through an Ih-mediated increase in input resistance. We also tested the effects of blocking inward-rectifying K+ currents, which are known to interact with Ih to affect resting membrane potential and synaptic excitability. Applying the K+ channel antagonist Ba2+ resulted in a similar increase in relative facilitation as was seen during block of Ih, but Ba2+ did not prevent further increases in the amplitude of train-evoked responses induced by ZD7288. This suggests that the Ih-dependent facilitation of train-evoked responses induced by carbachol is not dependent upon changes in input resistance associated with reductions in IKir. These findings emphasize the potent role of Ih in the integration of synaptic input to the entorhinal cortex during cholinergically induced oscillatory states.

Disclosures: D.W. Sparks: None. **C.A. Chapman:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.18/A27

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH-NIDA Grant DA003194

NIH-NIDA Grant DA015663

MNIRGDP-12-258900

SFARI 274443

NARSAD 21069

Title: Cell surface α4β2* nAChR expression is modulated by kinase activity and cholesterol content

Authors: *C. A. ZAMBRANO[1], C. A. SHORT[1], S. R. GRADY[1], M. J. MARKS[1,2], C. A. HOEFFER[1,3];
[1]Inst. for Behavioral Genet., Univ. of Colorado, BOULDER, CO; [2]Dept. of Psychology and Neurosci., [3]Dept. of Integrative Physiol., Univ. of Colorado, Boulder, CO

Abstract: Nicotinic acetylcholine receptors (nAChR) are ligand gated ion channels widely expressed in the nervous system. Chronic ligand exposure produces an increase (up-regulation) of receptor density in cellular and animal models and is observed in post-mortem brains of smokers compared to non-smokers. Even though the population of receptors expressed at the cell surface is up-regulated by chronic nicotine, their function may be maintained around basal levels because of receptor desensitization. Our previous data using primary mouse neuronal cultures chronically treated with nAChR antagonists such as mecamylamine and DHβE showed a specific effect on up-regulation of the surface α4β2* nAChR. This selective increase in surface binding differs from the pattern following chronic nicotine treatment which elicits increases in both surface and intracellular sites. Here we show that surface [125I]epibatidine binding (α4β2* nAChR), determined by a nAChR alkylation method, was decreased by both the cyclin-dependent kinase 5 inhibitor, roscovitine, and in a lesser extent by the protein kinase C inhibitors, chelerythrine and Go6983. Consistent with results obtained with PKC inhibitors, treatment with bryostatine, a PKC activator, specifically increased surface α4β2* nAChR expression. Interestingly, no effect of treatment with any of the kinase modulators was observed in the absence of nicotine treatment. In addition, the modulation of cholesterol content on the distribution of α4β2* nAChR was studied. Methyl-β-cyclodextrine was used to decrease cholesterol content of neurons in culture. Our results show a dose-dependent decrease of surface α4β2* nAChR when cells are treated with methyl-β-cyclodextrine; However, no changes in intracellular α4β2* nAChR were observed with this treatment. Our data suggest a mechanism for surface α4β2* nAChR expression that is phosphorylation-dependent. In addition surface expression is also sensitive to the levels of cholesterol in the cellular membranes. We speculate changes in cholesterol content impacts lipid domains such as lipid rafts. While this data cannot yet demonstrate a direct effect of those kinases and lipid rafts on the α4β2* nAChR, results do support the idea of a dynamic modulation of surface α4β2* nAChR that may affect receptor function. Contextual fear conditioned experiments in mice were also performed to determine the effect of withdrawal from chronic nicotine exposure on α4β2* nAChR expression and function. These studies are useful to better understand the behavioral consequences of α4β2* nAChR up-regulation.

Disclosures: C.A. Zambrano: None. **C.A. Short:** None. **S.R. Grady:** None. **M.J. Marks:** None. **C.A. Hoeffer:** None.

Poster

682. Brain Cholinergic Mechanisms

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM57481

Title: The molecular mechanism of the alpha7 nAChR silent agonist NS6740 is associated with nonconducting conformations of the receptor

Authors: *C. PENG, R. L. PAPKE;
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Abstract: The α7 nAChR silent agonist NS6740 has little efficacy for ion channel activation under control conditions. When co-applied with the α7 type II positive allosteric modulator (PAM) PNU-120596, NS6740 can selectively activate and/or desensitize α7 nAChR. After the application of high concentrations of NS6740 to α7-expressing cells, there was a striking reduction in the ACh control responses, suggesting that NS6740 induced a form of desensitization that was not readily reversible. We investigated the concentration and time dependence of NS6740's effects on α7 with and without 10 μM PNU-120596. Large responses to the co-applications of 10 nM to 3 μM NS6740 with PNU-120596 were observed, with a sharp decrease in the response when 10 μM NS6740 was co-applied with PNU-120596. ACh-evoked responses following the co-applications of PNU-120596 and NS6740 (<1 μM) were larger than the initial ACh controls, but after co-applications with NS6740 at ≥3 μM, there was a concentration-dependent decrease in the subsequent ACh controls. NS6740 acted as a competitive antagonist when co-applied with ACh, and the receptors remained in a desensitized state after washout. Preincubation with 3 μM NS6740 fully suppressed the response to the subsequent co-application of NS6740 and PNU-120596, indicating that NS6740 was effective at inducing and stabilizing the PAM-insensitive desensitized state (D_i) at levels of binding site occupancy at ≥3 μM. A single application of 30 μM NS6740 suppressed ACh-evoked responses for a prolonged period. An application of PNU-120596 immediately afterward showed that some receptors were in the PAM-sensitive desensitized state (D_s), but after 4 minutes all receptors converted to D_i. After 12 minutes, some receptors converted back to D_s, with the maximal PNU-120596 responsiveness observed after 20 minutes. Our studies suggest that NS6740 is a useful agent to probe the population of α7 receptors among the activatible and two forms of

desensitization states by using their PAM sensitivity. We also observed concentration-dependent modulation of synaptic function and long-term potentiation (LTP) in the rat dentate gyrus by NS6740 and the efficacious agonist nicotine. While 10 μM nicotine increased the magnitude of control LTP, 5 μM NS6740 inhibited control LTP that was co-incident with inhibitory effects on the baseline field excitatory postsynaptic potential (fEPSP) slope and peak of afferent volley, implying that fewer fibers were stimulated. Inhibitory effects on the control LTP and baseline fEPSP slope were increased by the addition of PNU-120596, indicating that the treatments selectively modulate GABAergic signaling.

Disclosures: C. Peng: None. **R.L. Papke:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.20/A29

Topic: B.02. Ligand-Gated Ion Channels

Support: Wellcome Trust 81925

Title: Varenicline binding interactions in 5-HT3 receptors are revealed by 5HTBP

Authors: *S. C. LUMMIS[1], R. K. LILLESTOL[1], K. L. PRICE[1], C. ULENS[2];
[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]KU Leuven and Xanelix, Leuven, Belgium

Abstract: Varenicline is a widely used and successful smoking cessation agent. Its primary therapeutic target is the nACh receptor, but it also acts at 5-HT3 receptors[1]. These latter interactions may be responsible for some of the side effects of varenicline (e.g. nausea) or even some of the less well-studied beneficial effects (e.g. decreased alcohol consumption[2]). The structure of varencline bound -AChBP has been published[3] but here we reveal the structure of varenicline bound to 5HTBP, a version of AChBP with a modified, 5-HT_3 receptor –like binding pocket[4]. To determine if this is representative of varenicline binding in the 5-HT_3 receptor, we explore the role of residues in the binding pocket of the 5-HT_3 receptor using mutagenesis , functional expression and radioligand binding. The data support the orientation of varenicline as seen in the 5HTBP structure, and suggest that 5HTBP is a useful protein with which to study interactions of drugs that act at the 5-HT_3 receptor. [1] Lummis et al., (2011) JPET 339:125-31 [2] Mitchell et al., (2012) Psychopharmacol. 223:299-306 [3] Billen et al., (2012) Proc Nat Acad.Sci. 109:9173-9178 [4] Kesters et al., (2013) EMBO Rep.14: 49-56.

Disclosures: **S.C. Lummis:** None. **R.K. Lillestol:** None. **K.L. Price:** None. **C. Ulens:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.21/A30

Topic: B.07. Synaptic Transmission

Support: G. F. Ribble-Biology (CM)

Title: The pharmacological profile and actions of cholinergic system in larval *Drosophila*: Behavior, development, CNS activity, and heart

Authors: ***C. MALLOY**, J. HILL, W. WU, R. L. COOPER;
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Abstract: We investigated the role of acetylcholine (Ach) on the *Drosophila* larval heart and CNS to identify its functional role and receptor pharmacology in this model organism. Genomic screens have revealed that there are ten receptors in *Drosophila* that are very similar to the nicotinic acetylcholine receptors (nAchRs) of mammals. In *Drosophila* acetylcholine is a transmitter within the CNS and is the neurotransmitter for sensory neurons but not motor neurons, as in mammals. A distinctive advantage of *Drosophila* larvae is the short developmental time (~4 days) in which the development of the CNS can be investigated. The alteration in neural activity related to circuits is particularly important during neural development for formation and stabilization of neural connections. In addition, the *Drosophila* larval heart offers a playground for future experimentation on the ionic regulation and modulation of pacemaker activity. We will report on the stimulatory effect of Ach on the larval heart and on a sensory-CNS-motor circuit. The significance of this study is presenting a testable model preparation for ion channels and Ca2+ transport function in regulating pacemaker potentials for cardiac cells and pacemaker cells as well as the pharmacological profile of AchRs in the CNS of the *Drosophila* model.

Disclosures: **C. Malloy:** None. **J. Hill:** None. **W. Wu:** None. **R.L. Cooper:** None.

Poster

682. Brain Cholinergic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 682.22/A31

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS069689

Title: Hippocampal cholinergic interneurons comprise a feed-forward excitation circuit

Authors: ***F. YI**[1], E. CATUDIO-GARRETT[1], R. GABRIEL[2], M. WILHELM[2], K. DEISSEROTH[3], J. LAWRENCE[1];
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Abstract: Acetylcholine (ACh) release in the hippocampus (HC) occurs during exploration, arousal, learning, and other cognitive processes (Pepeu and Giovannini 2004). Although the medial septum-diagonal band of Broca is the major source of cholinergic input to the HC, cholinergic interneurons intrinsic to the HC were discovered almost 30 years ago (Frotscher et al. 1986). However, HC cholinergic interneurons (HCIs) remain enigmatic and poorly understood. Here, we crossed ChAT-CRE mice and Rosa26YFP mice to yield ChAT-CRE/Rosa26YFP mice (ChAT-Rosa mice), enabling the visualization of EYFP+ HCIs. Histological examination of ChAT-Rosa mice revealed EYFP+ cells in each HC subregion. EYFP+ cells were more abundant in CA3 (19.4 ± 1.2, $p<0.0001$) and dentate gyrus (22.3 ± 1.0, $p<0.0001$) than CA1 (6.9 ± 0.4, avg. #/50 μm slice, n=88 slices, n=4 mice). Within CA1, there were more EYFP+ cells in stratum lacunosum moleculare (SLM; 2.5 ± 0.2, $p<0.0001$) and stratum radiatum (SR; 1.9 ± 0.1, $p=0.017$) than in stratum oriens (SO; 0.9 ± 0.1). Within CA3, EYFP cells were most abundant in stratum pyramidale (SP; 13.7 ± 1.0) compared to SO (1.3 ± 0.1, $p<0.0001$), SR (4.0 ± 0.3, $p<0.0001$), and SLM (0.4 ± 0.08, $p<0.0001$). Unlike cortical CIs (von Engelhardt et al. 2007), there was only modest overlap between EGFP+ (n=697) and VIP (n=7; 1.6%) cells, and calretinin cells (n=97; 13.9%), although overlap was higher in bipolar cells (VIP: 11.8%; calretinin: 39.8%). Upon current injection, firing properties were distinct between CA1 SR/LM (stuttering, 15/19) and CA3 SP (delayed, 15/15) HCIs, as well as passive properties (input resistance: 236.2 ± 13.8 vs. 143.8 ± 12.9 MΩ, $p = 0.036$; size: 111.6 ± 7.6 vs. 267.0 ± 16.9 pF, $p < 0.0001$, n=20, n=12, respectively). Upon wash-in of ACh, AP firing frequency was increased both in CA1 SR/SLM (by 86 ± 20%, $p= 0.0014$) and CA3 SP (by 20 ± 7%, $p = 0.0133$) HCIs. Relative to CA3 pyramidal cells, action potential half-width was wider in CA3 SP HCIs (1021.7 ± 38.0 vs. 725.8 ± 26.5 μs, $p = 0.0035$, n=12, n=7, respectively) and cholinergic modulation of AP firing was weaker (20 ± 7% vs. 115 ± 23%, $p= 0.0011$, n=11, n=7, respectively). Finally, consistent with the lack of co-localization of ChAT and GAD65/67 (Vida et al. 2000), optogenetic stimulation evoked glutamatergic EPSCs onto 6/7 SR interneurons. Therefore, HCIs constitute a feedforward excitatory circuit within the hippocampus.

Disclosures: **F. Yi:** None. **E. Catudio-Garrett:** None. **R. Gabriel:** None. **M. Wilhelm:** None. **K. Deisseroth:** None. **J. Lawrence:** None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 683.01/A32

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant 1R01DA037152-01

Title: Optically sensitive mu-opioid receptors for spatiotemporal dissection of pain and reward neural circuits

Authors: ***M. J. SCHMIDT**[1,2], M. BAIRD[1], W. PLANER[2], V. SAMINENI[3], B. COPITS[3], S. SPANGLER[1], R. AL-HASANI[2], J. G. MCCALL[2], R. W. GEREAU, IV[4], M. R. BRUCHAS[4]; [2]Anesthesiol., [3]Anesthesiology, Pain Ctr., [4]Anesthesiology, Pain Center, Anat. and Neurobio., [1]Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Opioids have well characterized actions in pain and addiction. However the microcircuitry of their effects in behaving animals has not been elucidated. We created optically sensitive mu opioid receptors (oMORs) to define the spatiotemporal dynamics of mu opioid signaling in pain and reward. These chimeric receptors join extracellular loops of rhodopsin, including criticial membrane domains for retinal binding, with intracellular components of mu opioid receptors. We cotransfected HEK293 cells with oMOR and pGloSensor-22F cAMP plasmid (Promega E2301). Light stimulation of oMOR expressing HEK293 cells suppressed forskolin-induced cAMP and activated pERK *in vitro* in a light power-dependent manner. Furthermore, oMOR demonstrated a distinct light duration-dependent pattern of ERK1/2 phosphorylation, indicating that the chimeric receptor signals similarly to an opioid receptor. Cellular trafficking assays showed that oMORs are expressed at the membrane, begin to internalize around 5min following light stimulation with peak internalization between 15-45min, and are recycled to the membrane after 60min which is remarkably similar to endogenous MOR activation with some agonists. oMORs were then packaged into an adenovirus vector (AAV5-EF1a-oMOR-WPRE) and injected into VGAT-IRES-Cre mice in brain structures relevant for mu opioid-driven behaviors such as conditioned place preference, a measure of reward. 5-7 weeks after injection, the ability of oMORs to modulate these regions in slice preparations or *in vivo* was recorded. AAV5-EF1a-oMOR-WPRE virus was expressed in the VTA and RMTg of

VGAT-IRES-Cre mice and was effectively trafficked to the membrane. In slice experiments, oMOR+ cells showed a robust, sustained, and reproducible GIRK current upon stimulation with 473nm laser light. *In vivo*, 473nm, 10mW light delivered through a fiber optic ferrule placed into VTA reward circuitry was sufficient to induce real time conditioned place preference. There was no effect of the oMOR virus being injected into VGAT-IRES-Cre (-) littermate controls or on VGAT-IRES-Cre (+) littermates injected with a control virus expressing eYFP only. Therefore, oMORs are light activated Gi-coupled GPCRs that signal and traffic in a similar fashion to endogenous mu opioid receptors and not rhodopsins. Furthermore, expressing and activating these receptors in the GABAergic neurons of the VTA and RMTg are sufficient to promote reward in mice. This new tool enables the dissection of neural pathways and behaviors long associated with mu opioid receptor activation with a level of cell type specificity not possible with pharmacological investigations.

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Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

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Program#/Poster#: 683.02/A33

Topic: B.03. G-Protein Linked Receptors

Support: NIH R21 DA034929

NIH R00 DA025182

NIH T32 DA007261

Title: Analysis of functional selectivity at the nociceptin opioid receptor

Authors: ***S. CHANG**[1], S. M. SPANGLER[1], S. W. MASCARELLA[2], G. E. MIGNECO[1], V. GUREVICH[3], F. I. CARROLL[2], M. R. BRUCHAS[1];
[1]Washington Univ. In St. Louis, St Louis, MO; [2]RTI Intl., Durham, NC; [3]Vanderbilt Univ., Nashville, TN

Abstract: The Nociceptin/Orphanin FQ Opioid Receptor (NOPR) is the most recently discovered and least characterized G-protein coupled receptor in the opioid receptor family. The

actions of NOPR and its endogenous ligand, nociceptin, have been thought to play a role in neuromodulation in the contexts of pain, opioid tolerance and addiction, as well as the modulation of stress and anxiety behaviors. It is thought that the NOP receptor, like other opioid receptors and GPCRs, may bind functionally selective ligands that can bias signal transduction, resulting in diverse behavioral effects. The potential for functional selectivity at the NOPR has yet to be explored. To quantitatively study the signal transduction profiles of NOPR ligands in-depth, we used real-time live-cell cAMP and Bioluminescence Resonance Energy Transfer (BRET) assays to study the ligand-induced Gα-protein activation and arrestin2/3 recruitment at NOPR. Additionally, we employed high-resolution confocal microscopy to identify differences ligand-induced receptor internalization. We screened multiple NOPR-selective ligands, as well as novel, custom-designed small molecules aimed at elucidating structural significance in signal transduction. We have found that the NOPR, like other opioid receptors exhibits different signaling profiles, depending on the ligand type. Furthermore, we show that minute changes in ligand structure can illicit opposing signaling profiles ranging from agonism to inverse agonism. Additionally, we propose plausible ligand-receptor docking configurations that may help to elucidate the relationship between conformation and signal transduction at the NOP receptor. Finally, we show that ligands are capable of eliciting different degrees of receptor internalization. Together, these data provide new and quantitative insight into the structural relationship between ligands and signal transduction at the NOP receptor, which will ultimately facilitate additional studies examining NOPR ligand bias in behavior. Supported by: NIH R21 DA034929, R00 DA025182 and T32 DA007261.

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Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 683.03/A34

Topic: B.03. G-Protein Linked Receptors

Title: Regulators of G-protein Signaling modulate chronic pain symptoms and responses to analgesic drugs

Authors: *S. GASPARI;
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Abstract: Chronic pain conditions lead to physical disability, they have a major impact on quality of life, and are often comorbid with major depression. Several chronic pain syndromes have been shown to involve substantial changes in functional and structural plasticity in the CNS. Regulators of G-protein Signaling (RGS) are intracellular proteins which module signaling duration and desensitization of several G-protein coupled receptors (GPCRs). Several members of the RGS family are expressed in the brain, and appear to function in a brain region and receptor/Galpha subunit selective manner. Previous studies from our laboratory have demonstrated the involvement of RGS9-2 in the modulation of opiate analgesia and tolerance in models of acute pain. Here we apply genetic mouse models to determine the role of Rgs9-2 and Rgs20 in inflammatory (formalin, CFA) and neuropathic pain (spared nerve injury). Rgs9-2 is expressed in the striatum, a brain region involved in affect and motivation, whereas Rgs20 is abundant in the periaqueductal gray, a key region for the modulation of nociception and analgesia. Our data reveal distinct roles of these proteins in chronic pain responses, as Rgs9-2 appears to modulate affective but not sensory components of chronic pain, whereas Rgs20 appears to play a potent role in inflammatory pain-like symptoms. In addition, we monitored chronic pain induced changes in gene and protein expression to further understand the cellular mechanism of Rgs9-2 and Rgs20 function and regulation. The last part of the study examined the role of these proteins in analgesic responses under chronic pain conditions. Our data reveal that while Rgs9-2 is a potent negative modulator of both opiate and tricyclic antidepressant responses, Rgs20 has such an effect only in morphine analgesia. These findings provide insight into brain region specific mechanisms modulating sensory and affective components of chronic pain, and point to RGS9-2 and Rgs20 proteins as promising new pharmacological targets for the treatment of chronic pain.

Disclosures: S. Gaspari: None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

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Topic: B.03. G-Protein Linked Receptors

Support: T32DA07278

DA35764

DA20570

Title: Arrestin-dependent and -independent mechanisms of mu and kappa opioid receptor inactivation

Authors: J. R. KUHAR, A. BEDINI, P. A. GROBLEWSKI, E. J. MELIEF, J. CHIU, *C. I. CHAVKIN;
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Abstract: The mu opioid receptor (MOR) agonists, morphine and fentanyl, both activate c-Jun N-terminal kinase 2 (JNK2), which is required for spinally-mediated morphine acute analgesic tolerance, whereas acute analgesic tolerance to fentanyl is blocked by G-protein receptor kinase 3 (GRK3) gene deletion. Similarly, the kappa opioid receptor (KOR) agonist, U50,488, and the KOR collateral antagonist, norBNI, stimulate phosphorylation of JNK, but JNK1 is specifically required for norBNI's long duration of antagonism. In this study, we tested centrally-mediated morphine analgesic tolerance in WT and JNK2-/- mice using the hotplate. We found that JNK2 is required for morphine tolerance in centrally-mediated pain circuits. WT and GRK3-/- animals were treated with saline or norBNI and challenged with U50,488 one week later before analgesia was assessed using the tail-flick assay. We found that norBNI's long duration of action was not blocked in GRK3-/- mice. Together, our *in vivo* data combined with previous work suggests that morphine and norBNI cause opioid receptor inactivation through a JNK dependent mechanism. To elucidate the molecular mechanisms of JNK activation, we treated JNK1-/-, JNK2-/-, JNK3-/-, and GRK3-/- mice with morphine and fentanyl 30-60min before harvesting spinal cord and analyzing phospho-JNK-IR by western blot. Additionally, MOR- or KOR-expressing HEK293 cells were transfected with siRNA for arrestin-2 or -3 or a scrambled siRNA control and 48hr later, cells were treated with drug and lysed for phospho-JNK-IR analysis. At MOR, we found that morphine activates JNK2 specifically in spinal cord and this activation was GRK3, arrestin-2, and arrestin-3 independent. Fentanyl also activates JNK2 specifically in spinal cord, but is GRK3 and arrestin-2 dependent. At KOR, we found that the early phase of U50,488-mediated JNK activation was arrestin independent, whereas the late phase was arrestin-2 dependent. Finally, norBNI-mediated JNK activation was arrestin-2 and -3 dependent. Our data suggests that MOR and KOR receptor inactivation occurs through a JNK mediated pathway and that JNK activation by MOR and KOR occurs through distinct arrestin-dependent and -independent mechanisms. These findings shed light on the molecular mechanisms of opioid tolerance providing knowledge that can be used to design opioids with reduced side effects.

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Poster

683. Opioid Receptor Signaling and Function

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Topic: B.03. G-Protein Linked Receptors

Support: CMUH Grants DMR-101-123 and DMR-102-029

NHRI Grants PD-102-PP-16 and NHRI-102A1-PDCO-1312141

Title: Alteration of adenylate cyclase activity in human embryonic kidney 293 cells coexpressing μ-, κ-opioid and nociceptin receptors after buprenorphine exposure

Authors: *C. W.-S. LEE[1], P.-C. WANG[2], I.-K. HO[1,3,2];
[1]Ctr. for Drug Abuse and Addiction, China Med. Univ. Hosp., Taichung, Taiwan; [2]Natl. Hlth. Res. Inst., Zhunan Town, Miaoli County, Taiwan; [3]China Med. Univ., Taichung, Taiwan

Abstract: Buprenorphine, used in maintenance therapy for heroin addicts, is a μ-opioid (MOP) receptor partial agonist and a potent κ-opioid (KOP) receptor antagonist as well as a nociceptin/opioid receptor-like 1 (NOP) receptor agonist. In this study, we established an *in vitro* cell model overexpressing human MOP, KOP, and NOP receptors individually or simultaneously in human embryonic kidney (HEK) 293 cells, and compared the effects of U-69593, DAMGO, nociceptin, and buprenorphine on adenylate cyclase (AC) activity in these cells (KOP, KOP+MOP, KOP+NOP, and KOP+MOP+NOP). After acute exposure, U-69593 inhibited AC activity in all four stable clones, showing that KOP receptor was successfully expressed. Acute application of DAMGO and nociceptin could elicit AC activity inhibition in cells expressing MOP and NOP receptors, respectively. Buprenorphine, when applied acutely, was able to inhibit AC activity to about 90% of the *E*max in cell expressing MOP, NOP and KOP receptors simultaneously. Chronic exposure to buprenorphine induced AC superactivation in cells coexpressing KOP and NOP receptors, and the level of AC superactivation was further elevated in KOP+MOP+NOP-expressing cells. The study demonstrated that MOP receptor might act as an enhancer in AC superactivation in HEK 293 cells coexpressing KOP, MOP and NOP receptors after long-term exposure to buprenorphine. **Acknowledgments:** This study was supported by the National Health Research Institutes (PD-102-PP-16 and NHRI-102A1-PDCO-1312141) and China Medical University Hospital (DMR-101-123 and DMR-102-029).

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Poster

683. Opioid Receptor Signaling and Function

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Program#/Poster#: 683.06/A37

Topic: B.03. G-Protein Linked Receptors

Support: NIH grant DA005010-27

NIH grant HD004612

Hatos Center for Neuropharmacology

Title: Opioid receptor responses of nucleus accumbens cholinergic interneurons

Authors: *D. NACHUN, C. CEPEDA, M. LEVINE, C. EVANS;
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Abstract: Cholinergic interneurons (CHIs) are the primary source of acetycholine (Ach) in the striatum and serve as critical regulators of its function, primarily via gating of excitatory inputs and inhibition of outputs. CHIs also express both mu- and delta-opioid receptors (MOR and DOR), unique among striatal interneurons. Despite this, very little is known about the role of CHIs in dependence to opiates and other drugs of abuse. Previous studies have indicated that manipulations of the cholinergic system in the nucleus accumbens have an effect on both conditioned place preference and self-administration of opiates. Recent evidence also indicates that upregulation of DORs on CHIs is important for natural reward learning. The present study was designed to characterize in more detail the responses of CHIs to MOR (DAMGO) and DOR (deltorphin) agonists. Slices containing the nucleus accumbens were prepared from 2-3 month old C57/BL6 mice. CHIs in the nucleus accumbens shell were identified using morphology and recordings were made using the cell attached configuration. All CHIs exhibited characteristic 1-3Hz spontaneous firing at baseline. As previously observed, bath application of MOR and DOR agonists reduced this firing rate. The DOR response was also observed to exhibit rapid desensitization after application of deltorphin. Experiments are currently ongoing to quantify any changes in MOR sensitivity after DAMGO application. Additionally, the independence of the MOR and DOR responses will also be examined by sequential application of DAMGO and deltorphin. For the second aim of the study, mice will be given chronic injections of morphine (every 12 hours for 4 days) and the effects of this treatment on CHI opiate responses will be examined using the same slice preparation, drug application and electrophysiology protocols. The response of CHIs to low (300 nM) and high (1 uM) doses of DAMGO and deltorphin will be examined. This experiment will help to indicate if chronic exposure to morphine produces persistent changes in sensitivity of MORs and DORs in CHIs. Future experiments will also seek to quantify changes in surface expression and sensitivity of MORs and DORs in CHIs and will also examine the behavioral significance of these opiate receptors. These experiments will help

to uncover if CHIs and the cholinergic system are an important part of the striatal circuitry which underlies opiate dependence.

Disclosures: **D. Nachun:** None. **C. Cepeda:** None. **M. Levine:** None. **C. Evans:** None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 683.07/A38

Topic: B.03. G-Protein Linked Receptors

Support: NIH MH-R01DA017204

Title: Further insights into the interaction mode of salvinorin A at the kappa-opioid receptor using molecular dynamics simulations

Authors: S. A. ZAIDI[1], B. L. ROTH[2], *P. D. MOSIER[1];
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Abstract: The mechanism by which the highly affine and non-basic agonist salvinorin A interacts with and modulates the function of the kappa-opioid receptor (KOP) is intriguing from a receptor-ligand interaction standpoint due to the nonbasic nature of the ligand. Previous hypotheses regarding the binding mode of salvinorin A and its analogs at KOP have relied on largely static homology models derived from related G protein-coupled receptors (GPCRs) or the recently-determined KOP crystal structure co-crystallized with the potent and long-acting antagonist JDTic (PDB ID = 4DJH). Here we report models of the unliganded and ligand-bound KOP in complex with either JDTic or salvinorin A in which conventional molecular dynamics (cMD) techniques have been employed to better understand the determinants of dynamic molecular recognition of these ligands by KOP. cMD simulations were performed with the KOP or KOP-ligand complex embedded in a phosphatidylcholine (POPC) lipid bilayer and explicitly solvated with water at a physiologically relevant ionic strength. The results show different conformational states of KOP depending upon the bound ligand. Notably, the conserved Asp3.32-Tyr7.43 interaction observed in most of the agonist- and antagonist-bound non-rhodopsin GPCR crystal structures (but not in 4DJH) is restored and maintained in the unliganded KOP. For the KOP-ligand complexes, both ligands retained their initial observed or experimentally-supported binding mode within the orthosteric binding cavity. Interestingly,

several structurally conserved water molecules were also identified whose position did not change during the course of the simulation for each ligand, supporting the role of water as an important determinant of molecular recognition of both charged and uncharged ligands by GPCRs.

Disclosures: **S.A. Zaidi:** None. **P.D. Mosier:** None. **B.L. Roth:** None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 683.08/A39

Topic: B.03. G-Protein Linked Receptors

Support: SCHA820/3-2

Title: Mechanisms of impaired sensory neuron opioid receptor efficacy during early stages of painful diabetic neuropathy

Authors: S. MOUSA[1], M. SHAQURA[2], B. KHALEFA[2], M. SHAKIBAEI[3], *M. SCHAEFER[2]; [1]Dep. of Anesthesiol & Intensive Care Med., Charité Univ. Berlin, Berlin, Germany; [2]Dep. of Anesthesiol & Intensive Care Medicine, Charité Univ. Berlin, CVK, Berlin, Germany; [3]Inst. of Anat., Ludwig-Maximilian-University Munich, Munich, Germany

Abstract: Several reports showed that painful diabetic neuropathic pain is a disease of the peripheral sensory neuron with impaired opioid responsiveness. However, the mechanism responsible for this reduced opioid efficacy during early painful diabetic neuropathy remaines elusive. Therefore, we investigated alterations of opioid responsiveness in streptozotocin-induced diabetic rats. In diabetic animals significantly impaired peripheral opioid analgesia was associated with a reduction in functional MOR G-protein coupling without significant changes in MOR density. MOR immunoreactive neurons increasingly colocalized with protein kinase C (PKC) alpha (α) isoform, activated forms of PKC as well as RAGE receptor during STZ-induced diabetes. Moreover, MOR phosphorylation at Thr370 in sensory neuron DRG of diabetic rats was due to RAGE-dependent PKC activation. Importantly, blocking PKC activation by PKC selective inhibitor, silencing RAGE recptor by intrathecal (i.t.) siRNA or preventing AGE formation by AGE inhibitor prevented sensory neuron MOR phosphorylation and consequently restored G protein coupling and antinociceptive efficacy. Thus, we unveil a tight association between MOR phosphorylation, RAGE-dependent PKC activation and peripheral sensory neuron

reduced opioid efficacy in diabetic rats. These findings demonstrate the therapeutic potential of impaired opioid responsiveness in early phase of diabetic neuropathic pain. Supported by DFG grant SCHA 820/3-2

Disclosures: **S. Mousa:** None. **M. Schaefer:** None. **M. Shaqura:** None. **B. Khalefa:** None. **M. Shakibaei:** None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

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Program#/Poster#: 683.09/A40

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant DA09082

Title: Morphine-induced trafficking of mu-opioid receptors in female locus coeruleus neurons

Authors: ***N. ENMAN**, B. A. S. REYES, E. J. VAN BOCKSTAELE;
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Abstract: The locus coeruleus (LC)-norepinephrine (NE) system is reciprocally regulated by the endogenous opioid enkephalin and the stress-related peptide corticotropin-releasing factor (CRF), which act postsynaptically via mu-opioid receptors (MOR) and CRF1 receptors located on noradrenergic neurons of the LC. Functionally, the LC is the sole source of NE to the forebrain and plays a major role in arousal and cognitive flexibility associated with the stress response. The LC-NE system is sensitized to CRF and stress following chronic morphine exposure, which is important as stress is a risk factor for the initiation and maintenance of opiate abuse. Additionally, recent evidence indicates enhanced female vulnerability to stress via CRF-related mechanisms in the LC compared to males. The exact mechanisms underlying enhanced sensitivity to stress following chronic opiate administration, and whether sex dictates the pattern of neural adaptations to chronic morphine, are presently unknown. In order to determine whether differences in expression levels or the subcellular distribution of MOR could partially underlie differences in opiate-induced sensitivity of the LC in males and females, localization of MOR was examined in the LC of morphine-treated male and female rats. As a first step, brains from naive male and female Sprague-Dawley rats were collected for Western blot analysis of MOR. Western blot analysis indicated that baseline MOR levels in naive animals are similar between sexes in this brain region. Next, male and female Sprague-Dawley rats were subcutaneously

implanted with two slow-release morphine (75 mg each) or placebo pellets over a five-day period, followed by transcardial perfusion with formaldehyde and the collection of brain tissue. Forty-micron thick coronal sections through the LC were processed for immunogold-silver labeling of MOR and were visualized using high-resolution immunoelectron microscopy. Placebo-treated male (0.37 ± 0.066) and female (0.33 ± 0.059) rats exhibited a similar ratio of cytoplasmic to total dendritic labeling for MOR. We confirm our prior studies showing MOR-induced internalization in male LC neurons (0.62 ± 0.066). Preliminary results indicate that morphine-implanted female rats exhibited a higher ratio of cytoplasmic to total dendritic labeling (0.56 ± 0.054) in LC compared to placebo-treated female rats, suggesting that morphine caused a modest shift in the distribution of MOR in females. These results indicate that morphine impacts MOR distribution in the LC similarly across males and females. **Support:** DA 09082

Disclosures: **N. Enman:** None. **B.A.S. Reyes:** None. **E.J. Van Bockstaele:** None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

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Program#/Poster#: 683.10/A41

Topic: B.03. G-Protein Linked Receptors

Support: Wellcome Trust UK

Title: Examining the roles of delta receptors and β-arrestin2 in mu receptor signalling in the VTA

Authors: ***F. BULL**[1], W. WALWYN[2], L. WRIGHT[1], T. G. HALES[1];
[1]Inst. of academic anaesthesia, Univ. of Dundee, Dundee, United Kingdom; [2]UCLA, Los Angeles, CA

Abstract: Mu opioid receptors located in the pain pathway mediate the analgesic actions of opioid agonists. Knock out mouse models reveal that opioids do not cause analgesia in animals lacking the μ opioid receptor. However, opioid analgesia is not diminished in mice that lack δ opioid receptors, and δ opioid receptor knockout mice exhibit reduced tolerance to morphine. Opioid receptor activation also leads to the recruitment of β-arrestin2, a scaffolding protein that interacts with various intracellular signal transducers. β-arr2-/- mice appear phenotypically normal but don't develop tolerance to opioid analgesia and show basal analgesia. Opioids also modify the affective component of pain by enhancing dopamine release in the mesocorticolimbic

pathway, a phenomenon associated with reward. They increase dopamine release by reducing GABAergic inhibition of dopaminergic neurones in the ventral tegmental area (VTA). The role of both the opioid receptors and beta-arrestin2 in the reward pathway remain largely unknown. We will characterise the actions of opioids on GABAergic neurones providing inhibitory input to dopaminergic neurones in the VTA. To do this we will utilise wild type (C57Bl6) mice, μ opioid receptor +/- and-/- mice, mice with a double knockout for both delta opioid receptors and beta-arrestin2 and mice that are +/- and -/- for β-arrestin2. We used the whole cell patch clamp technique to investigate the effects of opioid drugs on inhibitory postsynaptic currents (IPSCs) recorded from neurones within the VTA. Dopaminergic neurons in the VTA were identified using immunocytochemistry with an antibody against tyrosine hydroxylase. Bicuculline (10 μM) abolished spontaneous IPSCs (sIPSCs) recorded from VTA neurones without affecting tonic current. Morphine and the mu-selective peptide agonist DAMGO caused concentration-dependant inhibition of sIPSC frequency with EC_{50} values of 2.0 μM and 0.25 μM, respectively in wild type animals. Naloxone (10 μM) had no effect on the kinetics or frequency of sIPSC when applied alone, but reversed inhibition by opioid agonists. Reduced IPSC frequency reveals a presynaptic opioid inhibition of GABAergic neurones within the VTA. Disinhibition of dopaminergic neurones in the VTA is thought to be responsible for the dissociative effects of opioid analgesics. This action is helpful in the context of pain medication, but is also thought to be responsible for the abuse potential of morphine. An understanding of the mechanisms involved in opioid regulation of reward will help in the development better approaches to pain management.

Disclosures: **F. Bull:** None. **W. Walwyn:** None. **L. Wright:** None. **T.G. Hales:** None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

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Program#/Poster#: 683.11/A42

Topic: B.08. Synaptic Plasticity

Support: NS075278

NS080462-01

NS041218-10

Title: Opioid receptor signaling mediates homeostatic plasticity in the hippocampus

Authors: *B. N. QUEENAN[1], S. VICINI[2], D. T. S. PAK[2];
[1]Interdisciplinary Program in Neurosci., [2]Pharmacol. & Physiol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Neurons recalibrate synaptic signaling to stabilize neural networks via processes collectively known as homeostatic synaptic plasticity. In the mature hippocampus both *in vitro* and *in vivo*, homeostatic adjustment occurs at a designated "volume control synapse," namely the junctions between mossy fibers (MF) of granule cells in the dentate gyrus (DG) and thorny excrescences (TE) of CA3 pyramidal neurons. Chronic inactivity elaborates MF-TE synapses, while chronic hyperactivity dismantles these junctions. The functional elaboration of MF-TE is dictated by the activity state of DG granule cells. We wondered how the activity status of granule cells is encoded: how does a DG neuron know it has been "chronically inactive"? Mossy fiber terminals are selectively enriched in opioids, a family of presynaptic neuropeptides which includes dynorphin and enkephalin. We hypothesized that presynaptic opioid accumulation encodes the activity status of DG neurons and dictates the appropriate homeostatic response. We observed that opioids selectively and bidirectionally altered the strength of MF-TE synapses *in vitro* and that this regulation occurred through opioid receptor (OR) signaling. Kappa OR signaling promoted the upregulation of MF-TE synapses, while mu and delta OR signaling constrained MF-TE synapses. Moreover, kappa OR signaling was necessary and sufficient for the homeostatic upregulation of MF-TE synapses following chronic inactivity. These results suggest that presynaptic opioids control the MF-TE "volume control" synapse in the hippocampus. Dynorphin signaling through kappa OR signaling is known to be anticonvulsant, but dynorphin accumulation in MF terminals is depleted in many seizure models. Breakdown of kappa OR regulation of MF-TE homeostasis may therefore be a crucial component of the emergence of spontaneous seizures leading to epilepsy.

Disclosures: B.N. Queenan: None. **S. Vicini:** None. **D.T.S. Pak:** None.

Poster

683. Opioid Receptor Signaling and Function

Location: Halls A-C

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Program#/Poster#: 683.12/A43

Topic: B.03. G-Protein Linked Receptors

Support: UNE Startup Funds

Title: The dynorphins are functionally selective ligands at the mu opioid receptor

Authors: J. LAVIGNE[1], K. OLSON[2], F. PORRECA[2], *J. M. STREICHER[1];
[1]Biomed. Sci., Univ. of New England, Biddeford, ME; [2]Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: The opioid receptors (mu opioid receptor, MOR; delta opioid receptor, DOR; kappa opioid receptor, KOR) are major modulators of neuronal systems including pain, memory formation, addiction, and stress-induced relapse. These receptors are endogenously activated by a heterogenous family of opioid peptides, including the endorphins, dynorphins, and enkephalins. Various peptides have been associated with specific receptor targets and roles *in vivo*, such as dynorphin upregulation during stress states and mediating stress-induced relapse. However, *in vitro* studies have established that the majority of endogenous opioids have similar binding affinities to the MOR, DOR, and KOR. This raises the question of how these ligands with similar affinities at the same receptor can exert different functional effects. One potential explanation is functional selectivity of the peptides, whereby specific peptides could preferentially activate specific signaling cascades at the different opioid receptors, leading to specific *in vivo* roles. Utilizing [35S]-GTPγS coupling, βarrestin2 recruitment, and intracellular calcium flux (FLIPR) assays *in vitro*, we assayed potential functional selectivity of the endogenous ligands at the three opioid receptors in CHO cells. Focusing on the MOR, the results showed approximately equal potency and efficacy of all peptides in [35S]-GTPγS coupling, and endomorphin bias towards βarrestin2 recruitment, which has already been shown. Upon observing intracellular calcium flux, presumably a downstream second messenger of the βγ G protein subunit signaling, we observed an interesting bias. Dynorphin A and B became 10 fold less potent, the endomorphins became 10 fold more potent, and met- and leu-enkephalin stayed the same as in [35S]-GTPγS coupling. This suggests that the dynorphins have a bias towards the α subunit compared to the βγ subunits upon G protein activation from the MOR. Followup studies will examine the relative potency of the dynorphins in cAMP inhibition (α mediated) and DAG generation (βγ mediated), and study this functional selectivity in a more relevant neuroblastoma cell line (N2A expressing MOR). Overall these findings describe a novel type of functionally selective signaling, and may explain in part how peptides such as the dynorphins can have a specific role *in vivo* despite binding equally effectively to all opioid receptors. This new data may also lead to further understanding of how these ligands signal during normal and pain states *in vivo*, and give insight into the development of novel therapeutics for the treatment of chronic pain and related conditions.

Disclosures: J. LaVigne: None. **J.M. Streicher:** None. **F. Porreca:** None. **K. Olson:** None.

Poster

683. Opioid Receptor Signaling and Function

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Topic: B.03. G-Protein Linked Receptors

Support: Fondecyt grant # 1110352

MSI grant # P10/063F

Title: Effects of acute and repeated kappa opioid and dopamine D2 receptor activation on phasic and tonic dopamine neurotransmission in the nucleus accumbens

Authors: *A. P. ESCOBAR[1,3], M. P. GONZALEZ[1,3], J. A. FUENTEALBA[2,3], R. ESPAÑA[4], M. E. ANDRES[1,3];
[1]Cell. and molecular biology, [2]Pharm., Pontificia Univ. Catolica De Chile, Santiago, Chile; [3]Millenium Sci. Nucleus in Stress and Addiction, Santiago, Chile; [4]Neurobio. and Anat., Drexel Univ., Philadlphia, PA

Abstract: Repeated administration of the dopamine (DA) D2 receptor (D2R) agonist quinpirole (QNP) induces compulsive checking behavior and locomotor sensitization. Co-administration of U69593, a kappa opioid receptor (KOR) agonist, accelerates and potentiates QNP-induced behaviors. Both KOR and D2R locate presynaptically in the nucleus accumbens (NAc), and their individual activation decreases extracellular DA levels. Previous data show that repeated treatment with U69593 produces a loss of presynaptic D2R inhibitory control on DA release. We hypothesize that KOR controls D2 receptor function, resulting in the potentiation of QNP-induced locomotor sensitization. Our objective is to reveal the neurochemical and molecular interactions between these receptors in the NAc. Male Sprague-Dawley rats were repeatedly injected with U69593 alone or in combination with QNP, every two days until eight injections were completed. Horizontal locomotor activity was quantified for 60 min following each injection. Forty-eight hours after the eighth injection, rats were deeply anesthetized with chloral hydrate or urethane and subjected to *in vivo* microdialysis or fast scan cyclic voltammetry (FSCV) in the NAc to monitor tonic and phasic DA levels, respectively. During each experiment, rats were injected with U69593 followed by QNP at the same doses used for the pretreatment regimen. Another group of naïve rats were acutely injected with U69593 and/or QNP during the microdialysis or FSCV procedure. As expected, co-administration of U69593 significantly increases horizontal locomotor activity induced by QNP. Microdialysis data in naive rats showed that the acute activation of KOR blocks QNP-inhibition of K+-induced DA release but not basal extracellular levels. In addition, FSCV data showed that acute KOR activation accelerates QNP-induced decrease of phasic DA release. On the other hand, microdialysis data in rats repeatedly treated with U69593 and QNP showed that the presynaptic D2R inhibitory control on basal and K+-induced DA release is maintained. Taken together, our

results suggest that acute KOR activation differentially affects D2R function and reveal an indirect KOR control over D2R function in the NAc.

Disclosures: **A.P. Escobar:** None. **M.P. Gonzalez:** None. **J.A. Fuentealba:** None. **R. España:** None. **M.E. Andres:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 684.01/A45

Topic: B.04. Ion Channels

Support: NSF Grant DMS1121606

Title: Calcium buffers do not suppress (but may enhance) intrinsic free calcium concentration fluctuations in calcium microdomains

Authors: ***G. D. SMITH**, S. H. WEINBERG;
The Col. of William & Mary, Williamsburg, VA

Abstract: Intracellular calcium (Ca) plays a significant role in many cell signaling pathways, some of which are localized to spatially restricted "microdomains." Ca binding proteins (Ca buffers) typically slow [Ca] temporal dynamics and increase the effective volume of Ca domains. Because intrinsic fluctuations in [Ca] decrease in proportion to the square root of a domain's physical volume, one might conjecture that buffers decrease [Ca] fluctuations and, consequently, mitigate the significance of small domain volume vis-a-vis Ca signaling. We test this hypothesis through mathematical and computational analysis of idealized buffer-containing domains and their stochastic dynamics during free Ca influx with passive exchange of both Ca and buffer with bulk concentrations. We derive Langevin equations for the fluctuating dynamics of Ca and buffer and use these stochastic differential equations to determine the magnitude of [Ca] fluctuations for different buffer parameters (e.g., dissociation constant and concentration). In marked contrast to expectations based on a naive application of the principle of effective volume as employed in deterministic models of Ca signaling, we find that mobile and rapid buffers typically increase the magnitude of domain [Ca] fluctuations during periods of Ca influx, while stationary (immobile) Ca buffers do not. Also contrary to expectations, we find that in the absence of Ca influx, buffers influence the temporal characteristics, but not the magnitude, of [Ca] fluctuations. We derive an analytical formula describing the influence of rapid Ca buffers on [Ca] fluctuations and,

importantly, identify the stochastic analogue of (deterministic) effective domain volume. We also show how the theory of intrinsic free [Ca] fluctuations can be extended to account for spatial gradients in Ca "nanodomains" associated with individual Ca channels. Both the spatial and non-spatial theory demonstrate that Ca buffers alter the dynamics of domain [Ca] fluctuations in a non-intuitive manner. The finding that Ca buffers do not suppress intrinsic domain [Ca] fluctuations raises the intriguing question of whether or not domain [Ca] fluctuations are a physiologically significant aspect of local Ca signaling in neurons (e.g., dendritic spines). A manuscript focusing on the non-spatial theory of intrinsic domain [Ca] fluctuations has been accepted for publication in Biophysical Journal (Weinberg and Smith, 2014).

Disclosures: G.D. Smith: None. **S.H. Weinberg:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

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Topic: B.04. Ion Channels

Support: NIH R01HL71793

NIH R01HL54150

Title: Causal relationship between store-operated calcium entry, ER calcium stores, and ER stress/UPR in dopaminergic neurons

Authors: *A. YEN, C. OSLOWSKI, V. M. BOLOTINA;
Boston Univ. Sch. of Med., Boston, MA

Abstract: Store-operated Ca^{2+} entry (SOCE) is essential for the refilling of intracellular Ca^{2+} stores, but its relation to endoplasmic reticulum (ER) stress and role in degeneration of dopaminergic (DA) neurons is poorly understood. In this study, the human DA neuronal cell line (SH-SY5Y) was used to test the role of SOCE in the depletion of Ca^{2+} stores, ER stress, unfolded protein response (UPR), and cell viability, which may determine whether DA neurons will live or die. We found that the main components of SOCE (Orai1, STIM1, and PLA2g6) are present and fully functional in SH-SY5Y cells, and TG-induced depletion of ER stores results in significant SOCE. Interestingly, qRT-PCR revealed that mRNA expression levels of Orai1 and STIM1 were 50% lower than in HEK cells, while expression of PLA2g6 was 6-fold higher,

suggesting that PLA2g6 may compensate for the low Orai1 levels in DA neurons to ensure normal endogenous SOCE function. Further, we found that inhibition of Orai1 channels with diethylstilbestrol caused dramatic (92±2%) reduction in SOCE and caused depletion of Ca^{2+} in ER, as demonstrated by a 70±1% reduction in ionomycin-induced Ca^{2+} release in the presence of extracellular EGTA. Sustained inhibition of SOCE and depletion of ER Ca^{2+} resulted in significant ER stress and UPR, as manifested by 11±3 fold up-regulation of CHOP expression. Dose-dependent depletion of ER Ca^{2+} stores with increasing doses of thapsigargin (1-100nM for 24 hours) caused a dose-dependent increase in CHOP expression (up to 24±2 fold). Importantly, ER stress was observed only when more than 85% of the ER Ca^{2+} was depleted. Dose-dependency was consistent with ER Ca^{2+} depletion preceding and causing ER stress, with 1nM of TG needed to deplete 50% of Ca^{2+} from the stores, and 30 times more TG needed to achieve EC_{50} for CHOP up-regulation. Furthermore, a cell viability assay demonstrated that increase of ER stress and UPR induced by TG was closely followed by progressive loss of cell viability. We also found that TG-induced depletion of the stores and UPR can be rescued by molecular up-regulation of SOCE: overexpression of Orai1 channels resulted in more than 2 fold increase in SOCE, 85% increase of Ca^{2+} in ER stores, and about 50% attenuation of CHOP expression. Thus, there is a strong causal relationship between Orai1-mediated SOCE, ER Ca^{2+} stores and ER stress/UPR in the model DA neurons. Our results suggest that Orai1 and SOCE-dependent refilling of Ca^{2+} in the ER is essential for the prevention of ER stress, and impairment of SOCE may be a trigger for DA neuronal death and Parkinson's Disease.

Disclosures: **A. Yen:** None. **C. Oslowski:** None. **V.M. Bolotina:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

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Topic: B.04. Ion Channels

Support: Core Research for Evolutional Science & Technology, Japan Science and Technology Agency

Title: Recovery process from hydraulic stress-induced elevation of intracellular calcium is delayed with aging and accelerated by knockdown of Orai3

Authors: ***J. KUMAMOTO**[1], S. DENDA[1,2], K. TAKEI[1], M. GOTO[1,2], M. DENDA[1,2];
[1]Japan Sci. and Technol. Agency, CREST, Tokyo, Japan; [2]Shiseido Res. Ctr., Yokohama, Japan

Abstract: The uppermost layer of the skin, called epidermis, is mainly constructed with epithelial cells, called keratinocytes. At the bottom layer of the epidermis, keratinocytes proliferate and then move to the upper layer, accompanied with differentiation. Finally, they undergo apoptosis and contribute to formation of a water-impermeable layer, called stratum corneum. Various morphological and physiological changes appear in human epidermis with aging: stratum corneum becomes dry and flaky, while there is a decrease of keratohyalin granules, which produce free amino acids and cornified envelope. Moreover, the epidermal permeability barrier becomes more fragile and the recovery rate after barrier disruption became slower with aging. In young healthy epidermis, calcium ion concentration is higher in the uppermost layer of the epidermis, while the calcium gradation dissipates with aging. In our recently developed mathematical model of epidermal calcium dynamics, calcium channels and pumps play crucial roles. Thus, we hypothesized that the aging-associated change of calcium dynamics in keratinocytes might be due to altered expression of calcium ion modulators. We first observed the recovery process from hydraulic stress-induced intracellular calcium elevation in keratinocytes from a wide age range of subjects. The recovery tended to be delayed in keratinocytes from older subjects. We next evaluated mRNA expression of calcium modulators, focusing on members of the STIM-Orai system. When a Ca2+ decrease in endoplasmic reticulum is sensed by STIM1 on endoplasmic reticulum membrane, STIM1 formed clusters, which recruit and activate Orai1 to construct a calcium-permeable channel on the cell membrane. We found that the mRNA expression level of Orai3 was significantly correlated with age and inversely correlated with recovery rate of calcium ion concentration. Further, the recovery rate of cells from aged subjects was accelerated by knockdown of Orai3 with siRNA. These results suggest that aging-related changes in Orai3 expression are related to senile skin symptoms.

Disclosures: J. Kumamoto: None. **S. Denda:** None. **K. Takei:** None. **M. Goto:** None. **M. Denda:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

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Program#/Poster#: 684.04/A48

Topic: B.04. Ion Channels

Title: Characteristics of Ca2+ dynamics in single boutons of hippocampal fast-spiking and non-fast-spiking GABAergic interneurons recorded using femtosecond two-photon laser microscopy

Authors: T. LőRINCZ, *M. KISFALI, S. E. VIZI;
Dept. of Pharmacol., Inst. of Exptl. Medicine-HAS, Budapest, Hungary

Abstract: While a large amount of information, obtained using postsynaptic recordings, is available on GABAergic transmission in various hippocampal interneurons, the presynaptic Ca2+ signaling responsible for GABA release has remained almost fully uncharacterized. Our paper is the first attempt to provide a detailed analysis of presynaptic Ca2+ dynamics in fast-spiking (> 50 Hz) and non-fast-spiking (< 50 Hz) anatomically identified interneurons in the CA1 region of the rat hippocampus. We combined whole-cell patch-clamp recordings with two-photon scanning microscopy to record Ca2+ signaling in single boutons. This method (Kisfali et al., J. Physiol. 591: 5541-5553, 2013) offers the potential to study the mechanisms that govern the Ca2+ signals evoked by ongoing axonal activity. There was a significant difference in the amplitudes and shape of Ca2+ transients in response to somatic single or train stimulation recorded in non-fast-spiking (CB1 positive: Schaffer collateral-associated, apical dendritic innervating, perforant path-associated) and fast-spiking (parvalbumin positive: axo-axonic, basket, bistratified and parvalbumin negative: projection cells) interneurons pooled in separate groups. The non-fast-spiking neurons had much higher and longer-lasting transients than the transients observed in fast-spiking interneurons. The unperturbated values of Δ[Ca2+]i evoked by a single AP were 549 vs. 187 nM, and the unperturbated time constants of the decay were 245 vs. 141 ms in non-fast-spiking and fast-spiking interneurons, respectively. The [Ca2+]rest values were below 100 nM in both types of interneurons (73 vs. 68 nM). There was a difference in their endogenous Ca2+ buffering capacities (65 vs. 89) in non-fast-spiking and fast-spiking neurons, respectively. For the maximum [Ca2+], an approximately two-fold difference in the values of Δ[Ca2+]tot (35 vs. 17 μM) were evoked by a single stimulation. The analysis of the Ca2+ dynamics profiles revealed significant differences between the two types of interneurons. The fast-spiking GABAergic interneurons that synapse onto pyramidal cells have relatively short tails (fast recovery) of their Ca2+ transients, which are thereby able to promote short-lasting phasic inhibitory effects on pyramidal cell firing patterns and control the generation of oscillations in a short time window.

Disclosures: T. Lőrincz: None. **M. Kisfali:** None. **S.E. Vizi:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 684.05/A49

Topic: B.04. Ion Channels

Support: CREST-JST

MEXT

JSPS

Title: Spontaneous L-type calcium channel activity regulates local calcium signaling in the neurites of developing cortical neurons

Authors: *S. KAMIJO[1], K. SUZUKI[2], S. HORIGANE[1], H. FUJII[1], S. TAKEMOTO-KIMURA[1], H. BITO[1,3];
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Abstract: Recent evidence suggests that calcium levels in immature neurites are decoded by CaMK (Calcium/calmodulin-dependent kinase) I isoforms and regulate the elongation of axons or dendrites in a subtype-specific manner. However, the precise spatiotemporal dynamics of calcium signaling that controls these developmental events remains elusive. We here combined a novel membrane-tethered GCaMP indicator with *in utero* electroporation and performed extensive timelapse imaging of neurites of mouse immature cortical neurons that were destined to migrate into layer 2/3. We found that Na^+-spike independent Ca^{2+} transients arose locally and stochastically, in a manner that was highly unpredictable in location, spatial spread and time courses. Quantitative kymograph-based analyses, however, enabled extraction of memory effects in the temporal patterns of the Ca^{2+} elevations. To gain further insights on the molecular mechanisms of such variability and memory effect, we investigated the critical Ca^{2+} sources for these relatively slow spontaneous events in immature neurons. Spontaneous calcium elevations were significantly diminished by the addition of 10 μM nimodipine, a potent L-type calcium channel antagonist (cumulative $\Delta F/F_0$ (after/before): 52 %, n=27, $P<0.0001$) and 10 μM thapsigargin, a blocker of IP_3-dependent Ca^{2+} stores (47 %, n=8, $P<0.001$), but were insensitive to other voltage-gated channel blockers such as 10 μM ω-conotoxin MVIIC or 1 μM tetrodotoxin. ArcLight voltage imaging failed to reveal any detectable fluorescence change during spontaneous Ca^{2+} elevations. Furthermore, resting intracellular calcium levels were also altered by the addition of L-type calcium channel modulators. Immunocytochemistry using Western blot validated-antibodies confirmed that endogenous Cav1.2 and Cav1.3 channels were distributed in a scattered punctate manner. Consistently, “pharmacological knock-in” of nimodipine-insensitive Cav channels revealed that either Cav1.2 or Cav1.3 could contribute to calcium influxes across the plasma membranes and facilitate spontaneous Ca^{2+} transients. Together, these results suggest that L-type voltage-gated calcium channel activity critically regulate both spontaneous calcium elevation and maintenance of resting intracellular calcium level in developing cortical neurons, even in the absence of depolarizing Na^+ spikes.

Disclosures: **S. Kamijo:** None. **K. Suzuki:** None. **S. Horigane:** None. **H. Fujii:** None. **S. Takemoto-Kimura:** None. **H. Bito:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

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Topic: B.04. Ion Channels

Support: "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022014)" Rural Development Administration, Republic of Korea

Title: Cyanidin-3-glucoside inhibits ATP-induced $[Ca^{2+}]_i$ increase, formation of reactive oxygen species and mitochondrial depolarization in PC12 cells

Authors: *J. YANG[1,2], S. PERVEEN[1,2], S. YOON[1,2];
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Abstract: Cyanidin-3-glucoside is a member of the anthocyanin family and commonly found in pigmented fruits and vegetables. In this study, we determined whether cyanidin-3-glucoside affects ATP-induced calcium signaling using digital imaging methods for intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$), reactive oxygen species (ROS) and mitochondrial membrane potential. Treatment with 100 μM ATP for 90 s induced increases in $[Ca^{2+}]_i$. Pretreatment with cyanidin-3-glucoside (1 μg/ml to 100 μg/ml) for 30 min inhibited the ATP-induced $[Ca^{2+}]_i$ increases in a concentration-dependent manner (IC_{50}=15.28 μg/ml). Pretreatment with cyanidin-3-glucoside (15 μg/ml) for 30 min significantly inhibited the ATP-induced $[Ca^{2+}]_i$ responses following the removal of extracellular Ca^{2+} with Ca^{2+}-free solution or the depletion of intracellular $[Ca^{2+}]_i$ stores with thapsigargin (1 μM). Cyanidin-3-glucoside inhibited the ATP-induced $[Ca^{2+}]_i$ responses in the presence of nimodipine (1 μM). Cyanidin-3-glucoside also significantly inhibited 50 mM KCl-induced $[Ca^{2+}]_i$ increases. Moreover, cyanidin-3-glucoside significantly inhibited ATP-induced mitochondrial depolarization. In addition, cyanidin-3-glucoside significantly blocked ATP-induced formation of ROS. Therefore, the results suggest that cyanidin-3-glucoside inhibits ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells by inhibiting both the influx of extracellular Ca^{2+} and the release of Ca^{2+} from intracellular stores. In addition, cyanidin-3-glucoside inhibits ATP-induced mitochondrial depolarization and formation of ROS.

Disclosures: **J. Yang:** None. **S. Perveen:** None. **S. Yoon:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

Location: Halls A-C

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Program#/Poster#: 684.07/A51

Topic: B.04. Ion Channels

Support: NRF 2009-0083522

Title: Altered sleep pattern in mice with increased low-threshold burst firing in thalamocortical neurons

Authors: ***E. CHEONG**[1], J. LEE[2], J. HONG[1], H.-S. SHIN[2];
[1]Dept. of Biotech., Yonsei Univ., Seoul, Korea, Republic of; [2]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Daejeon, Korea, Republic of

Abstract: Sleep accompanies characteristic brain waves on electroencephalogram (EEG). The transition from wake to non-rapid eye movement (NREM) sleep state at the onset of sleep displays the replacement of low-voltage, fast frequency irregular EEG to large-amplitude, slow frequency EEG accompanying thalamocortical oscillations. The synchronized thalamocortical oscillations have also been implicated in paroxysmal spike-wave-discharges (SWDs), a hallmark of absence epilepsy. Although two distinct transitions from wake to loss of consciousness share a common brain circuit, the correlation between them remains unclear. Here we investigated the sleep pattern in phospholipase C β4 (PLCβ4)-deficient mice, absence epilepsy model with spontaneous SWDs by analyzing EEGs and EMGs during wake, NREM and REM sleep. We previously demonstrated a significant increase in low-threshold burst firing in PLCβ4$^{-/-}$ thalamocortical (TC) neurons. PLCβ4$^{-/-}$ mice displayed increased power density of delta waves during NREM sleep parallel with the increased NREM and total sleep amount. The occurrence of SWDs throughout different conscious states was also analyzed. These results indicate a crucial role of TC neurons in the generation of delta waves and stabilization of NREM sleep state.

Disclosures: **E. Cheong:** None. **J. Lee:** None. **J. Hong:** None. **H. Shin:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

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Topic: B.04. Ion Channels

Support: KAKENHI (22300127 to Y.K.)

Title: Enhanced CICR and SOCE in layer 3 pyramidal cells in the barrel cortex of PRIP-DKO mice

Authors: T. KAWANO, M. SAITO, H. TOYODA, H. SATO, *Y. KANG;
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Abstract: The two subtypes of phospholipase C-related but catalytically inactive protein (PRIP-1/2) are IP_3-binding proteins. IP_3-induced Ca^{2+} release (IICR) was impaired in cultured cortical neurons from PRIP-1 KO mice while store-operated Ca^{2+} entry (SOCE) was enhanced in hematopoietic B cells in PRIP-2 KO mice. In the present study, we aimed to clarify whether and how Ca^{2+} release from intracellular Ca^{2+} stores and SOCE are different between layer 3 (L3) pyramidal cells (PCs) in PRIP-1/2 double KO (DKO) and WT mice. The amplitude of Ca^{2+} transients was measured as a difference in the F_{340}/F_{380} ratio from the baseline level in L3 PCs in slice preparations incubated with fura-2 AM. A mixed solution of 20 mM K^+ and 20 mM caffeine induced large Ca^{2+} transients known as Ca^{2+}-induced Ca^{2+}-release (CICR), which decayed to a plateau level that persisted at least for 10 min examined. The mean peak and plateau amplitudes of CICR and the mean half-decay time from the peak CICR were significantly larger in PRIP-DKO PCs compared to the WT. We next examined whether SOCE occurred following CICR or not by reducing $[Ca^{2+}]_o$. The CICR was induced twice by the first and second applications of the caffeine/high-K^+ solution with an interval of 35-40 min. Immediately after the first and second CICRs, the caffeine/high-K^+ solution was changed to the extracellular solutions containing either 50 μM 2-aminoethyldiphenyl borate (2APB) or no and 2 mM Ca^{2+}, respectively. Compared to the decrease in the F_{340}/F_{380} ratio observed during application of the 2 mM Ca^{2+} extracellular solution after the second CICR, the ratio appeared to decrease more steeply during the application of the extracellular solution containing 2APB or no Ca^{2+} after the first CICR, and increased immediately after washout of 2APB or $[Ca^{2+}]_o$ was increased from 0 to 2 mM, indicating that CICR was followed by SOCE. Because SOCE occurs following CICR,

these results suggest that both the CICR and the subsequent SOCE are more potent in PRIP-DKO PCs than in WT PCs.

Disclosures: **T. Kawano:** None. **M. Saito:** None. **H. Toyoda:** None. **H. Sato:** None. **Y. Kang:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

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Topic: B.04. Ion Channels

Support: CIHR Grant MOP111211

Title: The characterization and role of mitochondrial Ca2+ dynamics in *Aplysia* neuroendocrine cells

Authors: ***C. J. GROTEN**[1], N. MAGOSKI[2];
[1]Physiol., [2]Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada

Abstract: In many neuronal types, mitochondria can influence cytosolic Ca2+ signalling during intense periods of activity. Consequently, these organelles stand to modulate Ca2+-dependent processes such as secretion and excitability. This property may be prominent in neuroendocrine cells, which transition from periods of relative quiescence to phases of high-frequency action potential firing to initiate hormone release. A tractable system for studying these processes is provided by the bag cell neurons of the marine mollusc, *Aplysia* californica. Upon stimulation, these neuroendocrine cells undergo a ~30 min period of sustained activity, known as an afterdischarge, during which intracellular Ca2+ is elevated to enhance excitability and hormone secretion, that in turn initiates reproduction. To study the contribution of mitochondrial Ca2+ handling during bursting, cultured bag cell neurons were fura-loaded with whole-cell recording, to monitor intracellular Ca2+ under voltage-clamp. Capacitance tracking was also used to monitor stimulus-evoked exocytosis and endocytosis of reproductive hormone. Mimicking the start of the afterdischarge with a 5-Hz, 1-min train of depolarizing steps caused an increase in cytosolic Ca2+ followed by Ca2+-induced Ca2+-release (CICR). The handling of Ca2+ influx was reduced by the protonophore, FCCP, which collapses the mitochondrial membrane potential, or double stranded RNA interference of either the mitochondrial Ca2+ uniporter or H+/Ca2+ exchanger, LetM1. CICR was dependent on mitochondrial Ca2+ extrusion, as shown its

sensitivity to low intracellular Na+ or pre-treatment with TPP (an inhibitor of mitochondrial Ca2+ exchangers). Furthermore, CICR was potentiated by inhibiting the plasma membrane Ca2+ ATP-ase with carboxyeosin or high-pH external. As the activity of voltage-gated Ca2+ channels are regulated by intracellular Ca2+, we determined the effect of mitochondrial Ca2+ buffering and release on Ca2+ currents. In the presence of FCCP, voltage-gated Ca2+ currents and Ca2+ influx were substantially reduced. Conversely, inhibiting mitochondrial CICR with TPP did not substantially alter Ca2+ currents. Lastly, inhibiting CICR with TPP did not alter exocytosis evoked by a train, but it accelerated the subsequent recovery of membrane capacitance. Collectively, we find a substantial contribution of mitochondrial Ca2+ uptake and release to bag cell neuron Ca2+ dynamics, which can regulate the activity of voltage-gated Ca2+ current and vesicle recycling. These results increase the known function of mitochondrial Ca2+ handling and demonstrate its importance to essential processes of the nervous system.

Disclosures: C.J. Groten: None. **N. Magoski:** None.

Poster

684. Calcium Channels: Intracellular Calcium Signaling

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Program#/Poster#: 684.10/A54

Topic: B.04. Ion Channels

Support: International Group of Neuroscience (independent sources)

Title: How can the open probability of ionic channels and the wave form of a burst be maximized by designing an EEG-triggered sequence of TES/TMS?

Authors: *J. F. GOMEZ-MOLINA[1], U. M. RICOY[2], I. ESCOBAR[3], J. VELEZ[4];
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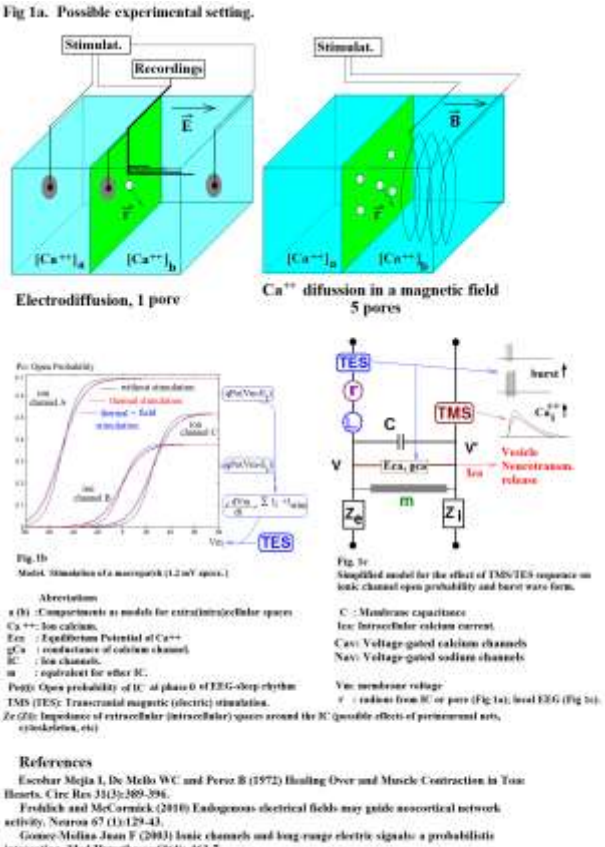
Abstract: INTRODUCTION. (i) *In vitro* experiments suggest that Ionic Channels (IC), like Cav2.1-2.3, have a frequency-dependent behavior in their responses to toxins and in their coupling to synaptic transmission (Ricoy and Frerking 2014). (ii) IC forms supramolecular structures and electrochemical couplings with other IC, the cytoskeleton, perineural nets, charged proteins, microdomains and transporters. These structures extend to extra and intracellular spaces. Theoretically they present new interesting properties: a. resonances (fn) of IC-

complexes; b. Electrodiffusion pathways beyond the IC-pore. This suggests that weak fields (endogenous or exogenous) at fn can affect selectively the open probability (Po(theta)) of certain IC-complexes in membranes, vesicles ER or nucleus. (iii) Ca2+ influx, electrical stimulation, temperature and [Ca2+]o increase the rate of healing in the heart (Escobar, DeMello and Perez 1972); (iv) Exogenous or endogenous fields that match the endogenous rhythms during sleep produces more effective effects (Frohlich and McCormick 2010). (v) TMS seems to act in nerve terminations. (vi) Po(theta) can be estimated from the EEG phase (Gomez-Molina 2003 2013a,b). METHODS. Math. Computer analysis. Simple experiments to test biophysical approximations (Fig 1a). RESULTS. We suggest that an initial short stimulation by TES can induce more Calcium influx (either opening Cav or Nav and increasing height and width of bursting) from the extracellular space. Immediately after, a TMS stimulation can stimulate intracellular flux of Calcium toward the vesicle. Thermal stimulation might determine noise level and parameters of fn (like amplitude). DISCUSSION. fn should also be specific (selective) to avoid secondary effects. CONCLUSIONS. (i) This stimulation is "friendly": it amplifies -instead of impose- the ongoing processes (IC-opening, Ca-influx, bursting, Cai) or the "healing reserve" (Opening of all IC -average= Pomax - Po(theta)). (ii) Similar considerations can be applied to the healing of the heart or the contraction of the muscle.



Disclosures: **J.F. Gomez-Molina:** None. **U.M. Ricoy:** None. **I. Escobar:** None. **J. Velez:** None.

Poster

685. Ca-Gated K Channels

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 685.01/A55

Topic: B.04. Ion Channels

Support: NIH

VA Merit Review

Title: Membrane properties and GABAergic synaptic transmission in central amygdala neurons from mice lacking BK channel beta-1 subunits: Implication of BK channels for alcoholism

Authors: *Q. LI[1], C. CONTET[2], S. TREISTMAN[3], S. MOORE[1];
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Abstract: The central nucleus of the amygdala (CeA) has been implicated in regulating alcohol drinking behavior and alcohol abuse. Alcohol may alter drinking behavior through its action on ion channels of neurons. Large conductance Ca++ -activated BK potassium channels are expressed in CeA neurons and regulate neuroal excitability and transmitter release. However, the role of BK channels and their accessory beta subunits in alcoholism remains unclear. Using whole cell patch-clamp recordings in an acute CeA slice preparation from genetically modified mice, we have examined membrane properties and inhibitory synaptic transmission in CeA neurons lacking BK channel beta-1 (BK-β1) subunits. We found no difference in resting membrane potential, time constants or input resistance between neurons from β1 knock-out (KO) and wild-type (WT) mice. Ethanol (50 mM) had no effect on time constants or action potential threshold in neurons from both KO and WT mice. However, neurons from KO mice fired more action potentials in response to depolarizing current injection than neurons from WT mice. In contrast to neurons from WT mice, action potentials in neurons from KO mice showed a stronger frequency dependent broadening with spike trains at 5, 10, 20 and 20 Hz. However, 50 mM ethanol caused a significant frequency dependent broadening in spike trains at 5, 10, 20 and 20 Hz in neurons from WT mice. Baseline frequency and amplitude of spontaneous postsynaptic inhibitory currents (sIPSCs) mediated by GABAA receptors were similar in neurons from KO and WT mice. Ethanol increased the frequency of sIPSCs in both groups without effects on amplitude of sIPSCs. Our preliminary results indicate that functional neuronal BK channels are present in CeA neurons lacking beta-1 auxiliary subunits, although beta-1 subunits may modulate ethanol sensitivity of BK channels.

Disclosures: Q. Li: None. **C. Contet:** None. **S. Treistman:** None. **S. Moore:** None.

Poster

685. Ca-Gated K Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: NHLBI R01-HL102758 (ALM)

NIAMS T32-AR007592 (JPW)

Title: Kcnmb2 is required for diurnal regulation of the BK K+ current and circadian patterning of neural activity in the mouse suprachiasmatic nucleus

Authors: *J. P. WHITT[1], A. L. MEREDITH[2];
[1]Physiol., Univ. of Maryland Baltimore, Baltimore, MD; [2]Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Circadian patterning of neural activity in the suprachiasmatic nucleus (SCN) of the hypothalamus, the brain's circadian clock, is achieved through daily regulation of ionic currents, including the BK K+ current. BK currents are larger at night, and the diurnal variation in BK current regulates SCN action potential (AP) frequency and circuit rhythmicity. Along with the BK α subunit (Kcnma1), two auxiliary β subunits, β2 (Kcnmb2) and β4 (Kcnmb4), are expressed in SCN, though not in a circadian manner. To assess the influence of β subunits on BK current properties and AP activity in the SCN, current- and voltage-clamp recordings were made from neurons in acute slices and circadian rhythmicity was assessed in organotypic cultures on a multi-electrode array. Knockout of β4 had no effect on SCN BK currents or circadian rhythmicity in AP frequency compared to wild-type (WT) controls. Conversely, knockout of β2 resulted in a loss of the day-night difference in BK current magnitude, reduced daytime AP activity, and diminished SCN circuit rhythmicity. β2, which confers inactivation to heterologously expressed BK channels, was required for inactivation of SCN BK currents, and inactivation could be restored by delivery of a soluble 45 amino acid peptide derived from the N-terminus of β2. The β2 peptide also reduced the daytime BK current and increased daytime AP firing in β2 knockout neurons to levels comparable to WT. These data suggest the β2 subunit plays a novel role in generating the diurnal difference in BK current magnitude, which is necessary for circadian patterning of AP activity in the SCN. Furthermore, the altered BK current magnitude and AP frequency in β2 knockouts can be rescued by exogenous delivery of the isolated β2 N-terminus.

Disclosures: J.P. Whitt: None. **A.L. Meredith:** None.

Poster

685. Ca-Gated K Channels

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: B.04. Ion Channels

Title: SK channel modulation attenuates alpha-synuclein-induced microglial activation

Authors: *A. M. DOLGA[1], F. WILHELMY[1], L. MATSCHKE[2], M. GOLD[3], G. GANJAM[1], R. DODEL[3], N. DECHER[2], C. CULMSEE[1];
[1]Inst. für Pharmakologie und Klinische Pharmazie, Philipps-Universität Marburg, Marburg, Germany; [2]Inst. of Physiol. and Pathophysiology, Univ. of Marburg, Marburg, Germany; [3]Dept. of Neurology, Univ. of Marburg, Marburg, Germany

Abstract: Small conductance calcium-activated potassium (SK) channel modulation is an emerging therapeutic approach for treatment of neurological diseases, including stroke, amyotrophic lateral sclerosis and neurodegenerative diseases, such as Parkinson's disease. Excitotoxicity, calcium deregulation, mitochondrial dysfunction and neuroinflammation contribute to progressive cell death in many neurodegenerative diseases. Our previous studies showed that activation of SK channels in neurons exerted protective effects through inhibition of NMDAR-mediated excitotoxicity. Further, we revealed recently that SK channels are also located at the inner mitochondrial membrane of neuronal mitochondria. In a model of glutamate toxicity, activation of SK channels attenuated mitochondrial fission, prevented the release of pro-apoptotic mitochondrial proteins, and reduced cell death. However, little is known about the function of SK channels in cell metabolism and neuroinflammatory processes in non-neuronal cells, such as microglial cells. In this study, we addressed the question whether SK channel activation affected inflammatory responses of primary mouse microglia upon α-synuclein challenge. We found that activation of SK channels significantly reduced activation of microglia in a concentration-dependent manner, as detected by real-time xCELLigence cell impedance measurements. Interestingly, α-synuclein-induced glycolysis was reduced by SK channel activation as detected by the cell metabolism Extracellular Flux Seahorse analyzer. Further data on cytokine (TNF-alpha and IL-6) analysis revealed that activation of SK channels attenuated α-synuclein-induced cytokine release. Inhibition of glycolysis prevented microglial activation and cytokine release. Although SK channel activation slightly reduced ATP levels, it attenuated α-synuclein-induced NO release. Overall, our findings show that activation of SK channels provides protective effects in microglial cells, likely via activation of both membrane and mitochondrial SK channels. Thus, SK channels are promising therapeutic targets for neurodegenerative disorders such as Parkinson's disease, where neuroinflammation and cell metabolic deregulation are associated with progression of the disease.

Disclosures: **A.M. Dolga:** None. **F. Wilhelmy:** None. **L. Matschke:** None. **M. Gold:** None. **G. Ganjam:** None. **R. Dodel:** None. **N. Decher:** None. **C. Culmsee:** None.

Poster

685. Ca-Gated K Channels

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 685.04/A58

Topic: B.04. Ion Channels

Title: An analysis of calcium dependent potassium current during an action potential from suprachiasmatic nucleus neurons

Authors: *J. R. CLAY;
Physiol., NIH, ROCKVILLE, MD

Abstract: The calcium ion current, ICa, and the Ca2+ dependent K+ current IKCA have been recorded from suprachiasmatic nucleus (SCN) neurons using voltage clamp steps for ICa and the action potential (AP) clamp technique for both ICa and IKCa (AC jackson, GL Yao, BP Bean, J. Neurosci. 24:7985, 2004). This study, which is a continuation of recent work on INa in SCN neurons (JR Clay, J. Neurophysiol. 110:2574, 2013), describes an analysis of the ICa and IKca results in which an experimentally recorded AP from an SCN neuron was digitized. The digitized data set was applied to mathematical models of ICa and IKCa in voltage-clamp mode. Those theoretical results were compared with experimental recordings of each respective component for model validation. The large conductance Ca2+ dependent K+ current, the BK channel, was used since this component is known to be present in SCN neurons. The analysis utilized the detailed measurements of the voltage and Ca2+ dependencies of BK from heterologous expression of these channels in Xenopus oocytes (J Cui, DH Cox, RW Aldrich, J. Gen. Physiol. 109:647, 1997). That work demonstrated that BK channels are present for all levels of intracellular Ca2+, Cai2+, including extremely low levels. In the later case large depolarizations of membrane potential are required to activate the channel. The primary role of influx of Ca2+ during an AP is to shift the activation curve of the BK channel to the range of membrane potentials spanned by an AP. The level of Cai2+ immediately following an AP remains significantly above the basal level in the model for several msec but the channel is not activated during that time because the membrane potential is below the range of channel activation even with elevated Ca2+. These results illustrate the role that BK channels may play during an AP in SCN neurons. The equations for BK will also be used, ultimately, in a model of the SCN AP, the long range goal of this work.

Disclosures: J.R. Clay: None.

Poster

685. Ca-Gated K Channels

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Topic: B.04. Ion Channels

Support: JSPS KAKENHI 23591265

JSPS KAKENHI 26461287

Title: Involvement of SK-type calcium-activated potassium channel in α-synuclein oligomer-mediated suppression of spike frequency in neocortical pyramidal neurons

Authors: *K. YAMAMOTO, H. SAWADA;
Clin. Res. Ctr. and Dept. of Neurol., Utano Natl. Hosp., Kyoto, Japan

Abstract: α-synuclein, which is a major component of Lewy bodies, has been proposed to play a central role in the pathogenesis of Parkinson's disease and dementia with Lewy bodies. Increasing evidence suggests that soluble oligomers rather than insoluble mature fibrils are the toxic species. The soluble α-synuclein oligomers could accumulate intracellularly and alter neuronal activities into pathological condition, but this hypothesis has not been studied directly. We examined the action of α-synuclein on neuronal excitabilities by introducing α-synuclein protein through whole-cell patch pipettes into pyramidal neurons in mouse frontal cortical slices. The following four kinds of internal solution were applied; α-synuclein (α-SN; 1μM), dopamine (DA; 10μM), α-synuclein co-incubated with dopamine at 37°C for 3 days (α-SN+DA), and vehicle solution. Western blot analysis indicated that the pipette solution including α-SN+DA had higher order oligomer in comparison with α-SN. Intracellular application of α-SN+DA significantly reduced spike firing during current injection, but did not changed resting membrane potential, spike width, and spike afterdepolarization, compared with other three kinds of solution. Nimodipine and apamin blocked the effect of α-SN+DA on spike firing. α-SN+DA prolonged the duration of spike afterhyperpolarization (AHP) and increased the charge transfer carried by the current elicited by depolarization pulses that would produce AHP under current clamp, both of which were inhibited by apamin. These results suggest that α-synuclein oligomers suppress spike frequency by enhancing AHP, and apamin-sensitive SK-type calcium-activated potassium channel is involved with the enhancement. This mechanism may cause the downregulation of neocortical activities in Parkinson's disease and dementia with Lewy bodies.

Disclosures: K. Yamamoto: None. **H. Sawada:** None.

Poster

685. Ca-Gated K Channels

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Topic: B.04. Ion Channels

Support: NINDS NS052574

REP Grant, UTHSCSA

Title: Reduced Kcnmb4 expression and changes in BK channel subtype in hippocampal granule neurons following seizure activity

Authors: *R. BRENNER[1], L. WHITMIRE[2], S. TIMILSINA[3], B. WANG[3], A. L. MEREDITH[4], D. B. JAFFE[5], J. CAVAZOS[3];
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Abstract: Changes in ion channel expression following brain insult and seizure activity can result in both adaptive and maladaptive changes in excitability. Large conductance voltage- and calcium-activated potassium (BK) channels contribute to early spike timing in hippocampal neurons, and several studies have indicated that the BK channel plays a pathological role in the maintenance of mesial temporal lobe epilepsy. In studies of BK channels two days following a pilocarpine-induced seizure, we have found that the predominant effect is a downregulation of the BK β4 accessory subunit. The result is a switch in the relative subtype of BK channels expressed; from iberiotoxin-resistant, type II BK channels (BKα/β4) that have a high channel open probability and slow gating, to iberiotoxin-sensitive type I channels (BKα alone) with low open probability and fast gating. Further, we report that BKα/β4 channels act to depolarize the action potential threshold. The switch to a majority of type I channel expression following seizure activity results in a loss of BK channel function on spike threshold while maintaining the channel's contribution to increased early spike frequency. We conclude that seizure-induced neuronal downregulation of Kcnmb4 is an activity dependent mechanism that increases the excitability of hippocampal granule cells.

Disclosures: R. Brenner: None. **L. Whitmire:** None. **S. Timilsina:** None. **B. Wang:** None. **A.L. Meredith:** None. **D.B. Jaffe:** None. **J. Cavazos:** None.

Poster

685. Ca-Gated K Channels

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 685.07/A61

Topic: B.04. Ion Channels

Support: CIHR

Title: Novel roles for IKCa channels in rodent hippocampal CA1 pyramidal neurons

Authors: *B. KING[1], A. P. RIZWAN[2], N. C. HEATH[2], H. ASMARA[2], S. N. DYKSTRA[2], G. W. ZAMPONI[2], R. W. TURNER[2];
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Abstract: Of the many classes of potassium channels recognized in central neurons, few have as key a role in regulating the frequency and pattern of spike discharge as calcium-gated potassium channels. Despite this, only two classes of calcium-gated potassium channels were previously recognized in the brain: big conductance (BK) and small conductance (SK) channels that generate AHPs to control excitability over time frames <50 ms. However, an unidentified class of calcium-gated potassium channel that generates a slow AHP (sAHP) that can last seconds exerts a far greater influence on the excitability of almost all neurons. We recently found that a sAHP in cerebellar Purkinje cells is generated by a third class of "intermediate conductance calcium-gated potassium channel" (IKCa) that was not believed to be expressed in central neurons. Our analyses reveal that IKCa channel immunolabel and KCNN4 promoter activity is widely expressed in other brain regions including CA1 pyramidal cells. We have used patch recordings in rat or mouse *in vitro* tissue slices to test the hypothesis that CA1 pyramidal cells express IKCa channels. Under conditions of BK, SK and Kv7 channel block we have recorded potassium current derived from calcium-activated channels of intermediate conductance that are sensitive to the IKCa channel blockers TRAM-34, Senicapoc, charybdotoxin, and maurotoxin, and enhanced by 1-EBIO, an agonist of IKCa channels. The sAHP is known to exert significant control over CA1 pyramidal cell excitability by restricting temporal summation of EPSPs and mediating spike accommodation. Internal infusion of 1 μM TRAM-34 blocked the sAHP evoked by repetitive stratum radiatum stimulation (5-30 pulses, 50 Hz) and recorded in the presence of apamin, XE-991, and picrotoxin to block SK and Kv7 channels and GABA receptors. TRAM-34 further blocked spike accommodation during synaptic trains or direct current injection in wild type but not KCa3.1 knockout animals. These data reveal the expression of IKCa channels in

CA1 hippocampal pyramidal cells that exert a significant contribution to the calcium-dependent component of the sAHP.

Disclosures: B. King: None. **A.P. Rizwan:** None. **N.C. Heath:** None. **H. Asmara:** None. **S.N. Dykstra:** None. **G.W. Zamponi:** None. **R.W. Turner:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: grant #CRP-26052 (L.R. 7/2007), 2010

P.O.R. F.S.E. 2007-2013 from Regione Autonoma della Sardegna

Title: Ethanol-dependent modulation of Ih currents in rat hippocampal CA3 pyramidal neurons: involvement in synaptic integration

Authors: V. LICHERI[1], J. DI LUCENTE[1], G. TALANI[2], G. SITZIA[1], G. BIGGIO[1], *E. SANNA[1];
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Abstract: It is well established that ethanol (EtOH), through the interaction with several membrane proteins, is capable of modulating many intracellular pathways and neuronal excitability. Recent studies reported that the hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels represent another sensitive molecule target for EtOH. Activation of HCN channels mediates a typical inward currents, termed Ih, which Ih play an important role in generating specific neuronal activities in different brain regions, including specific sub-regions of the hippocampal formation such as CA1 and CA3 being expressed in GLUergic pyramidal neurons and GABAergic interneurons. Ih contribute to control neuronal resting membrane potential and action potential discharge and are actively implicated in synaptic integration. Since robust Ih are also present in CA3 pyramidal neurons, we here have investigated whether the action of EtOH in the control of CA3 excitability can be correlated with its possible direct interaction with these cation channels. For this purpose, whole-cell patch-clamp experiments were performed in CA3 pyramidal neurons present in hippocampal coronal slices obtained from

juvenile male Sprague-Dawley rats. Voltage-clamp recordings revealed an Ih-evoked sag of the membrane potential (Vm) when incremental hyperpolarizing steps of the membrane voltage (from -65 to -115 mV) were given. When CsCl (5 mM) or the selective Ih blocker ZD7288 (20 μM) were bath-applied they completely prevented the generation of Ih-dependent Vm sag. Perfusion of EtOH at concentrations of 20-40 mM enhanced Ih-dependent Vm sag by about 25%, whereas 60-80 mM EtOH induced a significant and reversible reduction of this conductance by about 60%. Because Ih are involved in the control of synaptic integration, we evaluated if EtOH was able to modulate the temporal summation when a train of 4 stimuli at 20 Hz was given. In the presence of high concentrations of EtOH (60 mM) we observed an increase of summation, similarly to CsCl or ZD7288; on the contrary, 20 mM EtOH induced a decrease of this parameter. All togheter these data suggest that HCN channels, which mediates Ih currents in CA3 pyramidal neurons, are modulated by EtOH in a biphasic manner. This may represent an additional mechanism that contributes to EtOH's central action and neuronal excitability. This work was founded by a grant #CRP-26052 (L.R. 7/2007), 2010 and P.O.R. F.S.E. 2007-2013 from Regione Autonoma della Sardegna.

Disclosures: **V. Licheri:** None. **E. Sanna:** None. **J. Di Lucente:** None. **G. Talani:** None. **G. Sitzia:** None. **G. Biggio:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

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Program#/Poster#: 686.02/A63

Topic: B.04. Ion Channels

Support: NIH Grant SC1GM-084854

NIH Grant R25GM-061838

NSF Grant DBI-0932955

Title: Surface expression of the HCN2 subunit in Mesocorticolimbic areas after cocaine sensitization

Authors: *B. SANTOS VERA[1], A. M. RAMOS-CARDONA[2], A. C. VAQUER-ALICEA[1], R. VÁZQUEZ-TORRES[1], M. C. VELÁSQUEZ-MARTÍNEZ[3], C. A. JIMÉNEZ-RIVERA[1]; [1]Physiol. and Biophysics Dept., Univ. of Puerto Rico Med. Sci. Campus Sch. of Med., San Juan,

PR; [2]Psychology, Univ. of Puerto Rico Rio Piedras Campus, San Juan, PR; [3]Univ. Industrial de Santander, Bucaramanga, Colombia

Abstract: The Ih is a mixed cationic current present in excitable cells that is activated by hyperpolarization. This current acts as an important modulator of cell excitability in many brain regions. The Ih biophysical properties are largely determined by the expression profiles of the Hyperpolarization-activated cyclic nucleotide gated channels (HCN channels). These channels are widely expressed in Mesocorticolimbic (MCL) areas, especially the HCN2 subunit isoform. The MCL system is a set of interconnected regions that regulate pleasure, reward and motivation. However, even when there is a significant expression of HCN channels in the MCL system and DA neurons of the Ventral Tegmental Area (VTA) are identified using the presence of Ih, the HCN channels' role in addictive processes is largely unknown. Previously, we reported that cocaine-induced behavioral sensitization increases the HCN2 subunit total protein expression in all MCL areas. Also, we demonstrated a decreased Ih current in putative DA cells of the VTA after cocaine sensitization. In order to elucidate the relationship between these two findings, we investigated at what cell level is the increased HCN2 taking place. A protein cross-linking assay was used to distinguish between cell surface and intracellular localization of HCN2 subunits. Male Sprague Dawley Rats were sensitized to cocaine for seven days. Animals were treated with either 15 mg/kg i.p., cocaine hydrochloride or isovolumetric saline injections. Immediately after treatment, one hour of locomotion activity was recorded, once per day. Twenty-four hours after the last treatment injection, animals were sacrificed and brains removed. Tissue samples from the VTA, prefrontal cortex, nucleus accumbens, and hippocampus were taken. Samples were incubated in bis (sulfosuccinimidyl) suberate (BS3), used as a membrane-impermeant protein cross-linking reagent. The BS3 assay allows to differentiate between cell surface and intracellular protein pools. Afterwards, proteins were analyzed using Western Blots. Our findings demonstrate that the HCN2 surface/intracellular (S/I) expression ratios in the VTA were decreased after cocaine sensitization (sample T-test; $p< 0.017$) compared with saline treated animals. There was no difference in the S/I ratio for the HCN2 expression in the other areas of the MCL tested. We hypothesize that there is a possible disruption of the HCN2 channel's trafficking to the cell membrane, leading to less Ih current in this area after cocaine sensitization. Thus, the increased HCN2 subunit protein levels showed in the previous report can be due to a homeostatic process.

Disclosures: B. Santos Vera: None. **A.M. Ramos-Cardona:** None. **A.C. Vaquer-Alicea:** None. **R. Vázquez-Torres:** None. **M.C. Velásquez-Martínez:** None. **C.A. Jiménez-Rivera:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 686.03/A64

Topic: B.04. Ion Channels

Support: FAPESP

CAPES

Title: Nitric oxide modulates HCN channels in the supraoptic nucleus by s-nitrosylation-dependent mechanism

Authors: *M. P. SILVA, A. S. MECAWI, W. A. VARANDA;
Physiol., Sch. of Med. of Ribeirao Preto / Univ. of Sao Paulo, Ribeirão Preto, Brazil

Abstract: The electrical excitability of Magnocellular Neurosecretory cells (MNCs) of the Supraoptic Nucleus can be controlled by both synaptic inputs and intrinsic mechanisms. Several studies have shown that nitric oxide (NO) plays an important neuromodulatory role on these neurons, leading to inhibition in their activity during physiological and/or unbalanced conditions. Moreover, these responses can be independent of synaptic inputs suggesting a direct action of NO on ion channels. Thus, to investigate this hypothesis, hypothalamic slices (230 μm) and isolated MNCs from Wistar rats (80-100g) were prepared and membrane potential and hyperpolarization-activated cyclic nucleotide ionic currents (Ih) were measured with the patch clamp technique. Blocking HCN channels with ZD7288 (50 μM) decreases the spontaneous firing frequency of MNCs (Control = 4.3 ± 0.5 Hz; ZD7288 = 2.7 ± 0.5 Hz, n=6, $p<0.05$) indicating a reduction in their electrical excitability. This reduction in firing frequency was also previously observed by us when the cells were treated with NO. Therefore, the purpose of the present experiments was to analyze if the nitrergic modulation on MNCs occurs through an action on Ih currents. Our results show that 500 μM L-Arginine (substrate of NO) produced a significant reduction in Ih current (-12.3 ± 3 pA) in relation to control (-24.8 ± 1.3 pA; at - 130 mV, n = 6; $P < 0.05$). In contrast, 100 μM L-NAME (inhibitor of NO synthase) significantly increased Ih (from -26.5 ± 9.4 pA in control to -69.7 ± 15.8 pA; n = 6; $P < 0.05$). Inactive isomers (500 μM D-Arginine and 100 μM D-NAME) had no effects, confirming the nitrergic specificity. L-Arginine also decreased the Ih current in isolated MNCs (Control = -19.9 ± 5.9 pA/pF; L-Arginine = -6.8 ± 1.9 pA/pF, n=8; $p<0.05$ at -130 mV), and increased their activation time course (τ = 109 ± 6.2 ms in the control and τ = 167.8 ± 32.2 ms with L-Arginine, n=8, $p<0.05$). Our data also show that the effects of NO on Ih currents can be explained by s-nitrosylation of the HCN channels, since ODQ (specific blocker of guanylyl cyclase) did not change Ih. On the other side, NEM (blocker of s-nitrosylation - 300 μM) induced an increase in the current (-33.7 ± 10.1 pA vs -81.2 ± 13.4 pA, at -130 mV, n= 10, $P < 0005$). Additionally, L-

Arginine had no effects on Ih in the presence of NEM (-43.4 ± 9.4 vs -40.6 ± 6.8 pA at -130 mV, n=10, p>0.05). In conclusion, our results show that HCN channels are targets for the nitrergic modulation observed in MNCs of the supraoptic nucleus, which acts via s-nitrosylation mechanism.

Disclosures: **M.P. Silva:** None. **W.A. Varanda:** None. **A.S. Mecawi:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

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PHS NRSA T32 GM007270 from NIGMS

Epilepsy Foundation of America Pre-Doctoral Training Fellowship (189475)

Title: PKC bi-directionally modulates Ih and HCN1 surface expression in hippocampal principal neurons

Authors: *A. D. WILLIAMS[1], S. JUNG[2], N. POOLOS[2];
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Abstract: We and others have demonstrated the loss of expression and downregulation of gating of HCN channels in pyramidal neurons during the development of epilepsy following status epilepticus (SE) (Jung et al., 2007, 2010; Marcelin et al., 2009). The post-SE loss of dendritic HCN channel-mediated current (Ih) and HCN1 protein expression precedes the downregulation of HCN1 gene transcription, and thus may be due to post-translational mechanisms affecting HCN1 subunit trafficking to the surface membrane (Jung et al., 2011). We hypothesize that the early loss of Ih is due to changes in the phosphorylation state of HCN1 post-SE. There are no prior studies showing that HCN1-mediated Ih density is sensitive to phosphorylation in hippocampal principal neurons; we here sought to investigate phosphorylation as a mechanism regulating Ih density. Previously, we demonstrated serine phosphorylation as a mechanism controlling Ih density and HCN1 surface expression. Here, we considered Protein Kinase C (PKC) as a specific serine kinase controlling Ih density and HCN1 surface expression. We measured Ih at maximal activation at the soma of CA1 hippocampal pyramidal-like principal

(PLP) neurons from naïve rat brain slices using cell-attached patch clamp recordings and bath application of the PKC activator phorbol 12,13-diacetate (PDA). Following 30 min bath application of 10 μM PDA, Ih was diminished to 57 ± 8% of control ($p < 0.01$), with a -14 mV shift at the half-maximal value of Ih voltage-dependent activation. PDA also reduced the surface expression of HCN1 channels in hippocampal tissue from naïve rat brain slices (55 ± 11% of control, $p < 0.05$). Conversely, 60 min bath application of the PKC inhibitor GF 109203X increased Ih at maximal activation (158 ± 17% of control, $p < 0.005$). The increase in Ih occurred without a significant change in gating. Pretreatment with GF 109203X for 30 min followed by PDA application blocked the loss of Ih produced by PDA alone, producing Ih at maximal activation of 142 ± 18% of control ($p < 0.05$), comparable to bath application of GF 109203X alone. When application of PDA was followed by 60 min application of GF 109203X, Ih was 58 ± 15% control ($p < 0.05$) comparable to bath application of PDA alone, and showing that the loss of HCN1 surface expression caused by PKC activation was not rapidly reversible. These results indicate that Ih density and HCN1 surface expression can be bi-directionally modulated by PKC activity. The PDA-induced decrease in Ih was not rapidly reversible. These results support phosphorylation-mediated changes in HCN1 trafficking as a candidate mechanism underlying early changes in Ih density post-SE.

Disclosures: A.D. Williams: None. **S. Jung:** None. **N. Poolos:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 686.05/A66

Topic: B.04. Ion Channels

Support: UPR FIPI 2014-2015

Title: Ih current blocker ZD-7288 feeding to honey bees causes changes in sleep and learning behaviors

Authors: *E. J. RIVERA[1], T. GIRAY[2], J. L. AGOSTO[2], C. A. JIMENEZ-RIVERA[3];
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Abstract: Ih currents are mixed cation currents produced by Hyperpolarization-activated cyclic nucleotide channels (HCN). Ih currents are associated with the regulation of electrical activity in neurons, with effects including related to epilepsy and dopaminergic signaling. Honey bees represent a model where Ih current and behavior could be studied after pharmacological manipulations, since the honey bee HCN channel has been characterized for current kinetics, hyperpolarization activation, cyclic nucleotide regulation, and shown to be similar to the mammalian HCN channel. Biogenic amines like Dopamine (DA) (or Octopamine; OA) are known to modulate sleep and learning in insects. In order to study the effect of changes in Ih currents in the honey bee (*Apis mellifera,* sp.), we used ZD7288, an Ih blocker. We observed that indeed Ih blocker application is associated with changes in learning, memory and sleep behaviors of the honey bee. Using a place preference avoidance-conditioning assay, we found that changes in Ih causes significant changes in learning and does negatively influence short-term memory in honey bees. Also, using the proboscis extension assay, we want to know if changes in Ih affect appetitive learning. These results, taken together could support the hypothesis that Ih current is important for DA release and not necessarily OA. Using a modification of the *drosophila* activity monitoring (DAM) system for honey bees, we found that Ih blocker also has an effect on sleep patterns. Honey bees exposed to the Ih blocker ZD showed increased total sleep time and increased sleep fragmentation. The exposition to ZD caused a higher number of sleep episodes of a shorter duration compared to control groups. Out results on changes in learning and sleep caused by Ih blocker, suggests an interaction between Ih current and dopaminergic regulation.

Disclosures: E.J. Rivera: None. **T. Giray:** None. **J.L. Agosto:** None. **C.A. Jimenez-Rivera:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: NIH Grant 1R01MH090297-01A1

Title: Organotypic slice cultures of basolateral amygdala: A model for stress-related circuitry

Authors: *S. D. MICHAELSON[1], J. H. URBAN[2], W. F. COLMERS[1];
[1]Univ. of Alberta, Edmonton, AB, Canada; [2]Physiol. and Biophysics, Chicago Med. School, Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: While acute brain slice preparations have helped expand our understanding of the physiology and pharmacology of neuronal circuits, they are short-lived, precluding longitudinal studies. However, organotypic slice cultures (OTCs) not only retain the structure and connectivity of acute slices, but also permit controlled *in vitro* chronic studies. OTCs obtained from brain regions such as hippocampus and spinal cord conserve gross tissue organization, neuronal morphology and phenotype and connectivity, in common with age-matched acute slices of their respective regions, which validates this model. The amygdala is considered the principal regulator of emotional processing in the mammalian brain, and is the seat of one of the most prevalent psychiatric disorders, anxiety. Central to this role is a specific structure, the basolateral amygdala (BLA), which comprises glutamatergic pyramidal cells (80-85%) and GABAergic interneurons (15-20%). BLA pyramidal cells receive an array of inhibitory and excitatory inputs, and several neuromodulators regulate the activity of these neurons, including the anxiolytic neuropeptide Y (NPY). While we have established that the acute anxiolytic effect of NPY is mediated in part by a Y1-receptor action to reduce the tonic activation of the (excitatory) H-current (Ih) in pyramidal cells, NPY also plays a role in induction of long-term stress resilience. Rats injected with NPY into the BLA daily for 5 days show not only acute increases in social interaction (SI), but this effect also lasts up to 8 weeks. Slices prepared acutely from such animals show sharply diminished responses to CRF and to NPY, and reductions in Ih levels. To study this mechanistically, we have developed a novel OTC preparation of the BLA. Using P14 rats and the interface method, such cultures are viable up to at least 8 weeks and whole-cell patch recordings indicate that Ih is present in pyramidal cells of these OTCs and responds to acute application of NPY. We are further characterizing neuronal and synaptic properties and have

started to study the effects of prolonged exposure to NPY and NPY receptor-selective agonists on Ih in OTCs. Preliminary results suggest that prolonged exposure to NPY does not reduce Ih, however, pyramidal cell capacitance (directly proportional to cell surface area) is reduced and Ih activation kinetics are faster. We are reconstructing neurons to determine if pyramidal cell dendritic arbors are reduced with chronic NPY treatment. This OTC system will help to further our understanding of the cellular basis of stress resilience as well as of the signaling mechanisms downstream of NPY receptor activation that mediate this change.

Disclosures: S.D. Michaelson: None. **W.F. Colmers:** None. **J.H. Urban:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

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Topic: B.04. Ion Channels

Support: HFSP Grant (R.N.)

Microsoft Research India PhD Fellowship Award (M.S.)

Title: Subthreshold conductances regulate local field potentials and theta-frequency spike phase preference of hippocampal model neurons

Authors: *M. SINHA, R. NARAYANAN;
Mol. Biophysics Unit, Indian Inst. of Sci., Bangalore, India

Abstract: Despite the presence of a rich repertoire of somatodendritic subthreshold voltage-gated ion channels, the focus of most studies on local field potentials (LFP) has been confined to the contributions of synaptic and suprathreshold conductances. Can hyperpolarization-activated cyclic-nucleotide gated (HCN) channels and their somatodendritic gradients, given their ability to regulate intrinsic excitability and to introduce an inductive lead in impedance phase, alter LFPs and associated spike phase preference? Here, we computed LFP at different *strata* of the hippocampal CA1 region using line source approximation of currents from 400 morphologically realistic conductance-based models of pyramidal neurons. These neurons received randomized theta-frequency modulated excitatory inputs at dendrites and perisomatic inhibitory afferents. We inserted HCN channels into all model neurons with an experimentally constrained somatodendritic gradient and computed LFPs and neuronal spike phase preference. We

compared these measurements with those obtained in the absence of HCN channels, and observed a significant lead in the LFP phase across different *strata* of the CA1 region, without significant changes in LFP amplitudes. Further, the presence of HCN channels also resulted in a lag in the spike phase preference and increased spike phase coherence. As HCN channels alter both excitability and impedance phase, we assessed the relative contribution of these two components by replacing HCN channels with fast-activating counterparts and found a significant contribution of impedance phase to LFP and spike phases. How does plasticity in the synaptic receptors and HCN channels alter LFPs and spike phase? In answering this, we found that an increase in HCN conductance resulted in a progressive increase in LFP phase lead, spike phase lag and spike phase coherence. An increase in either excitatory or inhibitory synaptic conductances resulted in an increase in LFP amplitude, but did not significantly alter spike phase coherence. However, whereas an increase in excitatory conductances introduced a lag in the LFP phase and a lead in the spike phase, an increase in inhibitory conductances led to the opposite. Finally, on changing the phase difference between the theta-modulation of inhibitory and excitatory input afferents, we found that LFP and spike phases reliably reflected this difference. Our results suggest that subthreshold conductances can intricately regulate the LFPs and neuronal spike phase preference through changes in excitability and impedance phase, and identify specific roles for these conductances in phase coding and in the dynamics of cell assemblies.

Disclosures: **M. Sinha:** None. **R. Narayanan:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 686.08/B1

Topic: B.04. Ion Channels

Title: Primary adult rat microglia express Sur1-regulated channels

Authors: ***D. B. KURLAND**, N. CAFFES, J. K. KARIMY, V. GERZANICH, J. M. SIMARD; Neurosurg., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Microglia, the resident immune cells of the central nervous system, play a critical role in health and disease. Activated microglia, which can exert both neuroprotective or neurotoxic effects, undergo shape changes, proliferate, migrate toward stimuli and release active substances. Many of these programmed responses are tightly regulated by membrane potential and involve

precise, dynamic control of Ca2+ influx. Varying the expression of Sur1-regulated channels [KATP (Sur1-Kir6.2) and the non-selective cation channel Sur1-Trpm4], may be one mechanism by which activated microglia control Ca2+ homeostasis, as opening of KATP and Sur1-Trpm4 channels increases and decreases the driving force for Ca2+, respectively. To date, Sur1-regulated channels have yet to be identified specifically in microglia derived from adult tissue. Here, microglia were isolated from 3-4 month old naïve wistar rats using discontinuous percoll gradients and then plated in culture wells. The expression of KATP and Sur1-Trpm4 in resting and activated adult rat microglia was studied on the protein and mRNA level, via western blot/Co-IP and RT-PCR, respectively. The isolation procedure produced an essentially pure microglial population as >99% of cells immunolabeled for Iba1, a microglia/macrophage marker, and PCR amplification products for neuronal NeuN and astrocytic GFAP were absent. Microglia in culture developed a resting/quiescent phenotype after 18 hours, defined by ramified morphology and scant immunolabeling for the macrophage marker CD68/ED1. Upon stimulation with LPS (1µg/mL) for 24 hours, microglia adopted an activated phenotype, defined by morphological changes and increased immunolabeling for CD68/ED1. We observe that both resting and activated adult rat microglia express subunits of the KATP and Sur1-Trpm4 channels, albeit at different levels, suggesting that these channels may be involved in adult disease. We hypothesize that since these channels are intimately tied to membrane potential and Ca2+ influx, they may play an important role in regulating the programmed responses of activated microglia.

Disclosures: D.B. Kurland: None. **J.M. Simard:** None. **N. Caffes:** None. **J.K. Karimy:** None. **V. Gerzanich:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 686.09/B2

Topic: B.04. Ion Channels

Support: Acciones Integradas Hispano Húngaras (Ref. HH2006-0018)

Title: CNGA3 channels are expressed at the axonal initial segments in the CNS

Authors: *L. ROVIRA-ESTEBAN[1], A. I. MARQUÉS-MARÍ[1], Z. MAGLÓCZKY[2], N. HÁJOS[2], J. M. BLASCO-IBÁÑEZ[1];

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Abstract: Cyclic Nucleotide-Gated (CNG) channels are non-specific cation channels gated by direct binding of cyclic nucleotides cAMP or cGMP. CNG channels have a heterotetrameric structure consisting of α- and β-subunits. CNGA3 forms cGMP-sensitive functional channels in the cones of the retina, however, its occurrence in other parts of the CNS is unknown. To investigate the presence of CNGA3 in the brain, we used an antibody developed against the intracellular C-terminus of the protein using different fixatives in the rat. Although the labeling was similar with all of them, the levels of fine detail varied. Using the peroxidase method with nickel intensified DAB, the axon initial segments (AIS) were clearly visualized. The labeling was best in the sections fixed with glutaraldehyde or acrolein. Under light microscopy we could see that the expression of CNGA3 at the AIS of CNS was widespread. CNGA3-immunoreactive AIS were found in all checked areas: neocortex, cerebellum, the hippocampal formation, thalamus and hypothalamus. All neurons in these areas seemed to have CNGA3-immunoreactive AIS. In addition, both excitatory and inhibitory cells had CNGA3 immunoreactive AIS. For instance, in hippocampal CA1, where interneurons can be unequivocally distinguished based on the localization of their somata, all neurons outside the pyramidal layer had AIS immunoreactive for CNGA3. Also, in the cerebellum, GABAergic Purkinje cells had immunoreactive AIS. For electron microscopy, rat sections fixed with acrolein and human glutaraldehyde-fixed dentate gyrus sections were processed for the immunogold protocol. The nanogold particles were further subjected to silver enhancement. This methodology allowed subcellular detection of CNGA3. AISs were defined by their morphologic features such as undercoating, fascicules of packed microtubules and absence of rough endoplasmic reticulum that clearly distinguish them from dendrites. Silver particles appeared attached to the inner side of the AIS plasma membrane. This location corresponded with the location of the epitope inside the CNGA3 protein for which the antibody was designed against. CNGA3 has a motif to bind to the scaffolding protein ankyrin G that is abundantly expressed in the AIS. The presence of these non-selective cation channels at the AIS suggests that they are in the position to regulate the firing pattern of the neurons when the levels of cyclic nucleotides increase, as they do in sensory receptors.

Disclosures: L. Rovira-Esteban: None. **A.I. Marqués-marí:** None. **J.M. Blasco-Ibáñez:** None. **N. Hájos:** None. **Z. Maglóczky:** None.

Poster

686. Neuronal Excitability: Hcn and Non-Selective Cation Channels

Location: Halls A-C

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Program#/Poster#: 686.10/B3

Topic: B.04. Ion Channels

Support: NIH Grant NS77477 (D.J.)

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Title: Activation of inositol trisphosphate receptors is sufficient for inducing graded intrinsic plasticity in hippocampal pyramidal neurons

Authors: *S. ASHHAD[1], D. JOHNSTON[2], R. NARAYANAN[1];
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Abstract: A class of studies that endeavors to understand the role of store Ca^{2+} in neuronal plasticity employs pharmacological activation of metabotropic glutamate (mGluR) or acetylcholine receptors to mobilize cytosolic inositol trisphosphate ($InsP_3$). However, due to the intracellular presence of mGluRs, the diversity of signaling mechanisms downstream of these receptors, and the non-specificities associated with their pharmacological activation, the precise roles for Ca^{2+} released through $InsP_3$ receptors ($InsP_3R$) in neuronal plasticity has remained ambiguous. To overcome these limitations, we intracellularly injected D-myo-$InsP_3$ through whole-cell patch pipettes into rat (4–10 weeks male Sprague Dawley) hippocampal pyramidal neurons, and recorded several intrinsic properties for a 45-min period (at ~34 °C). We found that the injection of $InsP_3$ was sufficient to induce persistent plasticity of intrinsic response dynamics (IRD). Specifically, incorporation of 10 μM D-myo-$InsP_3$ in the recording pipette induced a reduction in input resistance (34.9 ± 6.8%) accompanied by an increase in the optimal response frequency (34.0 ± 5.7%) of these neurons. This plasticity in IRD also reflected as an increase in sag ratio, a reduction in temporal summation and an increase in the impedance phase lead. Strikingly, the magnitude of plasticity in all these measurements was dependent upon [$InsP_3$], emphasizing the graded dependence of such plasticity on the activation of $InsP_3R$. Assessing the mechanistic basis for this $InsP_3$-induced plasticity, we found that changes in all intrinsic response properties were abolished in the presence of ZD7288 (20 μM, pipette), establishing that this form of plasticity depended on hyperpolarization-activated cyclic-nucleotide gated (HCN) channels. Moreover, this Ca^{2+}-dependent (20 mM BAPTA, pipette) form of plasticity was critically contingent on the release of Ca^{2+} through $InsP_3Rs$ (1 mg/mL Heparin, pipette) and was modulated by the influx of Ca^{2+} through NMDA receptors (50 μM D,L-AP5, bath) and *T*-type Ca^{2+} channels (50 μM $NiCl_2$, bath). Finally, this form of $InsP_3$-dependent plasticity was reliant on the activation of the protein kinase A (PKA) pathway, evidenced by the blockade of plasticity

by a PKA inhibitory peptide (20 μM, pipette) or KT5720 (500 nM, bath). Our results suggest a critical role for the intracellular Ca^{2+} stores in regulating intrinsic properties under physiological conditions, thereby emerging as a pivotal cog in neuronal information encoding and homeostasis.

Disclosures: **S. Ashhad:** None. **D. Johnston:** None. **R. Narayanan:** None.

Poster

687. Acetylcholine and Other Transporters

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Program#/Poster#: 687.01/B4

Topic: B.05. Transporters

Support: NIH Grant R01NS38118 (D Sun).

Title: Inhibition of WNK3-SPAK/OSR1 kinase signaling reduces brain damage and accelerates neurological recovery following ischemic stroke

Authors: ***G. BEGUM**[1], S. WANG[1], K. T. KAHLE[2,3], L. LI[1], H. YUAN[1], Y. SHI[1], B. E. SHMUKLER[4], S.-S. YANG[5], S.-H. LIN[5], S. L. ALPER[4], D. SUN[1,6];
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Abstract: Derangements in ionic homeostasis contribute to cellular damage following ischemic stroke, but the molecular mediators underlying these processes are incompletely understood. The WNK (with no lysine = K) and associated downstream Ste20/SPS1-related proline-alanine-rich protein kinase (SPAK)/oxidative stress-responsive 1 (OSR1) serine-threonine kinases regulate the bumetanide-sensitive Na+-K+-2Cl- co-transporter (NKCC1) implicated in focal ischemia-induced neuroglial damage and death. However, WNK-SPAK/OSR1 pathway functions in normal and ischemic brain remain little understood. Here, we show that genetic deletion of WNK3, a WNK family member highly expressed in brain, or siRNA-mediated knockdown of SPAK or OSR1, protects against ischemic neuroglial death triggered by oxygen-glucose deprivation/reoxygenation. Protection is achieved via inhibition of ischemia-induced, stimulatory hyper-phosphorylation of NKCC1 at Thr203/Thr207/Thr212, a SPAK/OSR1 phospho-motif

regulating transporter activity. In WNK3 knockout mice subjected to focal cerebral ischemia by transient middle cerebral artery occlusion, normal ischemia-induced stimulatory hyper-phosphorylations of both the SPAK/OSR1 catalytic T-loop and NKCC1 Thr203/Thr207/Thr212 are abolished, infarct volume and axonal demyelination are reduced, and neurobehavioral recovery is accelerated. These data provide fresh insight into the roles of ion transporters and their regulatory kinases in ischemic neuroglial injury, and identify the WNK3-SPAK/OSR1 kinase complex as a compelling target for novel neuroprotective strategies following stroke.

Disclosures: **G. Begum:** None. **S. Wang:** None. **K.T. Kahle:** None. **L. Li:** None. **H. Yuan:** None. **Y. Shi:** None. **B.E. Shmukler:** None. **S. Yang:** None. **S. Lin:** None. **S.L. Alper:** None. **D. Sun:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: NIH Grant R01NS38118

NIH Grant P41EB-001977

Title: WNK3 KO mice exhibit reduced lesion size by ADC and DTI MRI after focal cerebral ischemia

Authors: X. GAO[1,2], L. M. FOLEY[3], *S. WANG[1], G. BEGUM[1], Y. SHI[1], L. M. FALGOUST[1], J. DENG[1], K. T. KAHLE[4,5], S. L. ALPER[6], T. HITCHENS[3], S. HU[2], D. SUN[1,7];
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Abstract: Kinases play an important role in the response to ischemic injury such as stroke. The WNK (with no lysine = K) and Ste20/SPS1-related proline-alanine-rich protein kinase

(SPAK)/oxidative stress-responsive 1 (OSR1) serine-threonine kinases comprise an evolutionarily conserved signaling pathway that regulates activities of multiple ion transporters and channels to control cell volume and epithelial ion transport. However, their roles in regulation of brain water homeostasis in ischemic brain remain unknown. Our recent study demonstrates that genetic deletion in mice of *WNK3*, a WNK family member exhibiting robust brain expression, is neuroprotective after transient focal ischemia (tMCAO). In this study, we conducted MRI analysis to investigate changes of brain edema and white matter injury in control (WT) and WNK3 knockout (KO) mice after tMCAO. The apparent diffusion coefficient (ADC) of brain tissue water measured by diffusion-weighted magnetic resonance imaging (MRI) can detect ischemic injury. Animals at 3 days post-ischemia were deeply anesthestisized (5% isoflurane in 70% N_2O/30% O_2), then perfused with PBS (pH7.4) and 4% paraformaldehyde in PBS (pH7.4). Ex-vivo brains (excised and stored in cold 0.1M PBS for 24-48 hrs) were imaged at 11.7T using a slice-selective spin echo-DTI sequence, with parameters that included 30 gradient directions, TR/TE = 2500/22 ms, b value = 1200 s/mm^2, 0.09 x 0.09 μm in-plane resolution, and Δ/δ = 10/5 ms, based on our previous studies. Infarct volumes were 13.8mm^3 ± 2.5mm^3 in WNK3 WT brains and 8.2mm^3 ± 3.9mm^3 in WNK3 KO brains, representing ~ 35% reduction in infarct volume in WNK3 KO mice. This is consistent with less edema formation in WNK3 KO brains, as determined by water content measurement. Diffusion tensor imaging (DTI) of fixed brains can reveal changes in structure, connectivity, and tissue integrity. Preliminary DTI results demonstrate no significant change in FA between the CL and IL corpus callosum in WNK3 KO mice (0.37mm^3 ± 0.03mm^3 vs. 0.35mm^3 ± 0.02mm^3, respectively) compared to a ~20% reduction found in WNK3 WT mice, (0.4mm^3 ± 0.03mm^3 vs. 0.32mm^3 ± 0.02mm^3 in the CL and IL corpus callosum, respectively). Taken together, our preliminary findings suggest that WNK3 transgenic knockout mice exhibit reduced grey matter damage and improved white matter integrity after ischemic insult.

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Poster

687. Acetylcholine and Other Transporters

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Program#/Poster#: 687.03/B6

Topic: B.05. Transporters

Support: Red de Universidades Públicas del Eje Cafetero Alma Máter

Title: Sodium-calcium exchanger (NCX) is regulated by leptin and insulin in rat cortical neurons

Authors: *J. C. SÁNCHEZ;
Facultad Ciencias de la Salud, Univ. Tecnologica De Pereira, Pereira, Colombia

Abstract: Introduction: Calcium can activate a number of cascades that may result in apoptosis and cell death. (1). The Na+/Ca2+ exchanger (NCX) is a bidirectional transporter of calcium in neurons and has been shown to be involved in neuroprotection, given that the NCX is a key factor in regulating the cytoplasmic concentration of this ion. This transporter is able to extrude calcium from the cell and may assist it in the recovery of cytosolic calcium levels after ischemia and excitotoxicity, thereby helping to decrease neuronal death in this process (2). Leptin and insulin are determinant in metabolism and have known neuroprotective effects (3, 4). Materials and methods: 7- to 10-day-old primary cultured cortical neurons from Wistar rats were subjected to patch clamp whole cell mode to record membrane currents (voltage clamp), using a holding potential (EH) of -40 mV, followed by a ramp pulse applied from +100 to -100 mV for 2 seconds at 0.5 Hz. The NCX blocker NiCl2 was added to the superfusing solution at the end of each experiment to obtain the Ni2+-sensitive current, representing INCX (Figure 1), in the presence of verapamil and ouabain. This current was inhibited by benzamil and KBR7943 and was regulated by extracellular and intracellular calcium. The leptin and insulin doses (100 ng/mL and 1 μM respectively) were those in which the maximal effect was observed in the respective dose-response relationship. Hormones were applied to the external solution immediately before the experiments. Additionally, a number of transduction signalling cascades (PKA, MAPK NOS, PI3K and PKC) inhibitors were employed to determine their effects. Results: Both insulin and leptin increased the inward currents (calcium extrusion mode, to -80 mV 44±7%, n=8, $p<0.05$ y 36.2±11%, n=8, $p<0.05$, respectively) and decreased the outward currents (calcium entrance mode, +80 mV 31±7%, n=8, $p <0.05$ y 21.3±6%, n=8, $p<0.05$, respectively). The effect of insulin was inhibited by queleritrin, which suggest that it is mediated by proteinkinase (PKC), but not for the other signaling pathways inhibited. On the other hand, the effect of leptin was not affected by any of the inhibitors employed. Discussion: Both leptin and insulin increased the inward component of INCX and decreased the outward currents; in other words both hormones decreased Ca2+ influx and increased Ca2+ efflux mediated by NCX, which can help to reduce neuronal death secondary to different noxious agents when these hormones are employed. These effects on the NCX could be a mechanism explaining the neuroprotective actions of these two hormones, and these findings could help researchers understand the role of the NCX in neuroprotection.

Disclosures: J.C. Sánchez: None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Title: Neuronal ClC-3 splice variants exhibit similar biophysical properties but differ in the subcellular localization in hippocampal neurons

Authors: *R. E. GUZMAN, E. MIRANDA-LAFERTE, T. GENSCH, M. COMINI, C. FAHLKE;
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Abstract: ClC-3 is a member of the CLC family of anion channels and transporters that localizes to early and late endosomes as well as to synaptic vesicles. Its ablation in genetically modified mice results in pronounced hippocampal and retinal degeneration suggesting a critical role of ClC-3 in the CNS. We recently demonstrated that ClC-3 functions as a presynaptic transporter and controls synaptic strength by regulating the amount of neurotransmitter as well as the release probability of synaptic vesicles (Guzman et al. (2014) Front Cell Neurosci DOI: 10.3389/fncel.2014.00143). However, others have proposed anion channel function and postsynaptic localization for ClC-3 (Wang et al (2006) Neuron, 52, 321-333). Since ClC-3 is alternatively spliced and it might result in distinct proteins with diverse functions and subcellular localization we decided to compare the biophysical properties and cellular distribution of neuronal ClC-3 splice variants. We performed RT-PCR from RNA extracted from mouse hippocampal tissue and identified, cloned and sequenced three different splice variants, ClC-3a, ClC-3b and ClC-3c. Expression of these splice variants in HEK293T cells revealed exclusive localization of ClC-3a and ClC-3b in intracellular compartments. In contrast, ClC-3c is not only found in intracellular compartments, but also in the plasma membrane. ClC-3a contains a single clathrin-binding motif in its amino terminus, and its disruption results in surface membrane insertion. We identified two additional clathrin-binding motifs with similar function in the amino-terminal region of ClC-3b. Heterologous expression of mutant ClC-3a/b and wild type ClC-3c in HEK293T cells resulted in the occurrence of coupled Cl-/H+ exchangers. For all splice variants, Cl- currents are outwardly rectifying and exhibit comparable time and voltage dependences. They exhibit significant capacitive current components indicating that there is a high probability of non-complete transport cycles under our conditions, as described for the previously characterized ClC-3short (Guzman et al. (2013) ACS Chem Neurosci 4, 994-1003, DOI: 10.1021/cn400032z). We used lentivirus and confocal microscopy in hippocampal neuronal culture to deliver and visualize the subcellular localization of the ClC-3 wild type

proteins. ClC-3b and ClC-3c exhibit similar dotted appearances along the neuronal processes, whereas ClC-3a localizes to the cell body with limited expression to dendrites/axon structures. Our results demonstrate that alternative splicing results in ClC-3 transporters with similar biophysical properties but separate subcellular distribution in hippocampal neurons.

Disclosures: R.E. Guzman: None. **E. Miranda-Laferte:** None. **T. Gensch:** None. **M. Comini:** None. **C. Fahlke:** None.

Poster

687. Acetylcholine and Other Transporters

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Support: NIMH Grant MH092797

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Title: *In vitro* and *in vivo* characterization of [^{11}C]TZ659 and [^{3}H]TZ659 for vesicular acetylcholine transporter

Authors: H. LIU[1], H. JIN[1], X. ZHANG[1], J. LI[1], K. KANESHIGE[2], S. M. PARSONS[2], J. S. PERLMUTTER[1], *Z. TU[1];
[1]Washington Univ., SAINT LOUIS, MO; [2]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Cholinergic dysfunction plays a key role for cognitive deficiency in neurodegenerative pathology. Vesicular acetylcholine transporter (VAChT), which loads acetylcholine into presynaptic vesicles, is regarded as a reliable cholinergic biomarker. A positron emission tomography (PET) tracer for imaging VAChT would provide an opportunity to noninvasively quantify VAChT *in vivo*, could serve to evaluate cholinergic function in patients with neurodegenerative diseases and could monitor potential therapeutic effects. Our group recently developed a promising radioligand ([^{11}C]**TZ659**) for VAChT. Herein we reported *in vitro* and *in vivo* characterization of this novel radiotracer and its H-3 labeled counterpart [^{3}H]**TZ659**. *In vitro* binding characterizations were performed using the PC12$^{A123.7}$ cell line transfected with human VAChT gene and rat brain tissue via [^{3}H]**TZ659**. *In vivo* binding assays, the bio-distribution study, as well as *ex vivo* autoradiography using [^{11}C]**TZ659**, were performed

to determine *in vivo* binding specificity. A saturated binding curve was observed from the *in vitro* saturation binding assay, with K_d = 1.97 ± 0.30 nM and B_{max} = 3240.00 ± 145.90 fmol/mg protein. Competitive binding studies of [^{3}H]**TZ659** were performed using σ_1, σ_2, vesicular monoamine transporter 2 (VMAT2), dopaminergic, serotonergic, or phosphodiesterase 10A (PDE10A) specific ligands, and known VAChT inhibitors to inhibit binding of [^{3}H]**TZ659**. Only VAChT-binding compounds (K_i ranged from 0.20 nM to 31.35 nM) inhibited the binding of [^{3}H]**TZ659.** *In vitro* binding study of [^{3}H]**TZ659** with rat brain homogenates showed high specific binding in the striatum compared with cerebellum demonstrating a high signal-to-noise (S/N) ratio (S/N ratio >3.46). Higher S/N ratios were observed using *in vivo* binding study (S/N ratio = 9.56 ± 1.11) and bio-distribution study (S/N ratio as 3.64 ± 0.72, 5.53 ± 1.88 for 30 and 60 min post-injection respectively) using [^{11}C]**TZ659**. *Ex vivo* autoradiography of [^{11}C]**TZ659** confirmed it has high specificity in the striatum, with a consistently high S/N ratio (S/N = 2.99 ± 0.44 at 30 min post-injection). In conclusion, this study revealed that [^{11}C]**TZ659** has nanomolar high affinity and good specificity for VAChT *in vitro* and *in vivo*. These data suggest that radio-labeled [^{11}C]**TZ659** is a promising PET tracer to image VAChT in the brain. Further investigation of this tracer will lead to translational clinical investigation of this promising PET radiotracer for quantifying the level of VAChT in brain to assess cholinergic denervation in patients with neurodegenerative pathology.

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Poster

687. Acetylcholine and Other Transporters

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 687.06/B9

Topic: B.05. Transporters

Support: NIH COBRE Grant to DSU

Title: Effects of changes in acetylcholine release on locomotion states in central synapses

Authors: A. BLAKE[1], N. KENDALL[1], S. BOPPANA[1], T. KITAMOTO[2], *H. O. LAWAL[1]; [1]Biol., Delaware State Univ., Dover, DE; [2]Anesthesiol., Univ. of Iowa, Iowa City, IA

Abstract: Cholinergic dysfunction plays a role in addiction to nicotine, and neurological diseases such as Alzheimer's disease, as well as the decline in cognitive function seen in normal

aging. The key elements required for presynaptic acetylcholine (ACh) release and post-synaptic signaling are known. However, the relationship between alterations in their function and downstream changes in behavior in the nervous system remain poorly understood. One critical element of cholinergic signaling is the vesicular acetylcholine transporter (VAChT) which is required for the transport of ACh from the cytoplasm into synaptic vesicles for exocytotic release. A complete loss of vacht is lethal in flies, worms and mammals. Here we hypothesize that subtle changes in will uncover important roles of ACh release in behavior. Indeed, we report for the first time the effect of three vacht point mutations on baseline and touch response locomotion paradigms. In particular, we note that vacht mutants have a deficit in timing of response to touch stimulus; and based on this phenotype, we categorized the mutants into three classes, mild, moderate and severe. This result suggests a differential deficit in acetylcholine release in these mutants. Further, we report genetic and pharmacological rescue of the touch response timing deficit using a wildtype VAChT insertion and the dopamine agonist pergolide, respectively. Moreover, we show a characterization of a selection of these "rescued" animals. Together, this report demonstrates a key role for acetylcholine signaling in both baseline locomotion and the timing of the response to a mechanical stimulus and underscores the utility of point mutations that compromise VAChT activity *in vivo* as a tool to elucidate the complex relationship between altered ACh release and behavioral deficits.

Disclosures: A. Blake: None. **N. Kendall:** None. **S. Boppana:** None. **T. Kitamoto:** None. **H.O. Lawal:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: NIMH-SCI MH086070

DA 029989

2T34GM008048

Title: Mapping glycinergic circuitry in the adult rat basal ganglia

Authors: *R. ORTEGA, M. PANDO, P. LOZANO, B. LOPEZ, M. MIRANDA-ARANGO; Biol., Univ. of Texas at El Paso, El Paso, TX

Abstract: The basal ganglia controls the initiation of motor movements and responses that involves cognitive functions such as emotion, vision and some forms of memory. The structures of the basal ganglia include the striatum, globus pallidus (GP), subthalamic nucleus, entopeduncular nucleus, and substantia nigra (SN). Among these, the GP is enriched in GABAergic nerve terminals that regulate voluntary locomotor activity. Of interest, Parkinson's disease is a degenerative disorder of the basal ganglia due to the loss of dopaminergic neurons and irregular pallidal electrical discharges. GABAergic inputs to dopaminergic neurons are vital for the sensory modulation of these nerve cells through the expression of GABAA and GABAB receptors. Conversely, glycinergic nuclei and fibers were believed to be located in caudal regions of the CNS; however, more recent studies demonstrate the presence of these inhibitory neurons in subcortical forebrain regions. Interestingly, these glycinergic fibers are involved in the control of coordinated motor functions such as breathing, audition and vision, among others. Although it is known that GlyT1 is abundant in the spinal cord, brainstem and retina, we have identified strong GlyT1 immunoreactivity in several areas of the forebrain. We performed immunohistochemistry to characterize the cell type and brain areas of GlyT1 expression. Retrograde and anterograde tracing experiments and microscopy analysis of sagittal rat brain sections revealed the presence of GlyT1 in cells from the basal ganglia, cerebellum and brain stem. This data likely indicates a possible relationship between glycinergic and dopaminergic neurotransmission in voluntary motor activity. The function of these glycinergic circuits will be studied using optogenetics combined with electrophysiology and behavior. These experiments will provide the precise location of neuronal circuits containing glycine and identify the behaviors they regulate.

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Poster

687. Acetylcholine and Other Transporters

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Program#/Poster#: 687.08/B11

Topic: B.05. Transporters

Title: N-terminus determines the targeting of K+-Cl- co-transporter KCC2 to neuronal plasma membrane

Authors: P. FRIEDEL[1], A. LUDWIG[2], C. PELLEGRINO[1], C. RIVERA[2,1], *I. MEDINA[1]; [1]INSERM, Marseille, France; [2]Neurosci. Ctr., Helsinki, Finland

Abstract: The activity of neuronal potassium-chloride co-transporter KCC2, which is critically involved in neurological disorders, is determined primarily by the rate of its surface insertion/internalization. While recent works identified regions on the KCC2 involved in mechanism of pathology-induced transporter internalization, the structural determinants controlling its plasmalemmal insertion remain unknown. Here, using KCC2 construct harbouring pH-sensitive tag (pHluorin) in the 2nd extracellular loop, we identified the cytoplasmic N-terminus as an indispensable region for the transporter trafficking into the neuronal plasma membrane. The deletion of this domain fully abolished the surface insertion of the transporter, whereas mutant composed of N-terminus and KCC2 transmembrane domains (deletion of C-terminus) effectively targeted cell surface, but showed a higher rate of internalization. Our finding changes the current view on the structure-functional role of cytoplasmic regions of KCC2 and highlights the N-terminus as potential target for strategies directed to restore transporter function following neurological disorders.

Disclosures: P. Friedel: None. **A. Ludwig:** None. **C. Rivera:** None. **I. Medina:** None. **C. Pellegrino:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

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Fondecyt grant 1141132

SQM 2014

Title: Evans Blue blocked stimulated but not basal release of ATP from sympathetic nerve terminals, suggesting different control mechanism of secretion

Authors: *R. A. BARRA[1,2], B. CAYUPE[2], M. V. DONOSO[2], J. P. HUIDOBRO-TORO[2]; [1]Univ. De Santiago De Chile, Santiago De Chile, Chile; [2]Dept. de Biologia, Lab. de Nucleotidos, Univ. de Santiago de Chile, Santiago de Chile, Chile

Abstract: Evans Blue (EB) interferes with the vesicular nucleotide transporter (VNUT) blocking vesicular ATP uptake; we reasoned that EB should reduce the ATP released from sympathetic nerve endings. To assess this hypothesis we examined whether EB inhibited the release of vesicular ATP. We evaluated basal and stimulated overflow of ATP, noradrenaline (NA) and neuropeptide Y (NPY), following electrical depolarization of mesentery nerves. To this aim, the isolated rat arterial mesenteric bed was perfused *ex vivo* and the release of co-transmitters, ATP, NE and ir-NPY, induced by field stimulation (60V, 20Hz, 1msec for 1 min) of these nerves was quantified in the buffer perfusate from control and mesenteries perfused with 0.1 or 1 µM EB. Parallel groups were treated with 0.2mg or 2mg/kg reserpine for 48h. The total release of ATP, NA and ir-NPY in control experiments was 77.17±18.74 (pmol, n=12); 35.46±11.68 (pmol, n=7) and 67.61±14.42 (fmol, n=12), respectively. In the tissues added 0.1 or 1 µM EB, the released ATP was 33.97±16.07 and 4.47±1.72 (n=5 each, $P<0.05$ for the latter); however, the ATP released with 0.1µM EB, was not significantly different from basal values. In the same tissues, NA released was 14.09±2.26 and 2.69±0.42 (n=5-6 each, $P<0.05$ for the latter), while ir-NPY was 33.96±12.37 (n=5) and 38.59±12.58 (n=3), respectively. EB did not alter basal co-transmitter released. 0.2mg/kg Reserpine reduced ATP to: 6.27±3.83, NA to 14.63±5.01 (n=3 each, $P<0.01$), and ir- NPY to 89.97±25.17 (n=3). Increasing reserpine to 2mg/kg, ATP released was 27.59±7.3 (n=5), NA was 0.10±0.09 (n=5, $P<0.01$) and ir-NPY was 7,79±2,37 (n=4, $P<0.01$). Reserpine reduced only basal release of NA. The 20Hz stimuli increased perfusion pressure (PP) in controls by 131.8±14.3 % of 70 mM KCl contracture (n=9); EB did not modify PP. Reserpine 0.2 or 2mg/kg decreased PP to 48.2±17.7 (n=3) and 13.3±8.2 (n=5, $P<0.01$). In sum; EB did not reduce basal release of co-transmitters. In addition, 0.1 µM EB selectively reduced the outflow of ATP induced by electrical stimulation. In contrast, reserpine dramatically reduced both basal and stimulated NA overflow and it also ir-NPY released. Since EB did not modify PP, we propose that ATP might not have a direct contractile role in this tissue.

Disclosures: R.A. Barra: None. **B. Cayupe:** None. **M.V. Donoso:** None. **J.P. Huidobro-Toro:** None.

Poster

687. Acetylcholine and Other Transporters

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Support: R01-DK081567

R01-NS018400

5T32HL007913-13

Title: The anion exchanger 3 is essential for the robust acidification response in CA1 hippocampal neurons during metabolic acidosis

Authors: A. I. SALAMEH, *W. F. BORON;
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Abstract: Objective: One of the main acid loaders in CA1 hippocampal neurons is the anion exchanger 3 (AE3). It acid loads the neurons by facilitating the exchange of extracellular Cl^-; for intracellular HCO_3^-. In the present study we investigate the significance of AE3 activity during low-[HCO_3^-] metabolic acidosis (MAc) in CA1 neurons. Methods: (1) To study AE3's role in the recovery from intracellular alkalosis, we induced an alkaline load, bathing the neurons in 10% CO_2 / 44 mM HCO_3^- at pH 7.4, and then switching to 5% CO_2 / 22 mM HCO_3^- at pH 7.4. (2) To study AE3's role in extracellular MAc, we bath the neurons in 5% CO_2 / 22 mM HCO_3^- / 130 mM Cl^-, at pH = 7.4 for 10 min. We then applied MAc solution (5% CO_2 / 14 mM HCO_3^- / 130 mM Cl^-, at pH = 7.2) for 7 min. Finally, we applied a Cl^--free MAc solution for another 7 min. We obtained our data by fluorescence imaging using the pH sensitive dye BCECF. Results: Our results show that (1) in the intracellular alkaline load experiments, the rate of the recovery from the alkaline load ($dpH_i/dt_{alkaline}$) at intracellular pH (pH_i) 7.4 is reduced by 54% in $AE3^{-/-}$ neurons. (2) In the MAc experiments, there is no significant difference in the initial steady-state pH_i between WT and $AE3^{-/-}$ neurons. (3) In the WT, MAc induced a robust acidification rate ($dpH_i/dt_{acid} = -1.3 \times 10^{-3}$) followed by small stabilization (slope = -1.1×10^{-4}). In the $AE3^{-/-}$, dpH_i/dt_{acid} is decreased (-9.5×10^{-4}) and pH_i continues to drift (slope = -4.2×10^{-4}). (5) The decrease in pH_i at the end of the rapid MAc response ($\Delta pH_{i\text{-}fast}$) is significantly reduced in $AE3^{-/-}$, whereas the decrease in pH_i at the end of the 7 min ($\Delta pH_{i\text{-}end}$) is not significantly different. (6) When we remove extracellular Cl^- in the WT neurons, pH_i increases rapidly to higher levels; however, in $AE3^{-/-}$ the response to Cl^- removal is slower. Conclusions: The decrease in $dpH_i/dt_{alkaline}$ in $AE3^{-/-}$ neurons indicates that AE3 is a major acid loader in CA1 neurons during intracellular alkalosis. The reduction in dpH_i/dt_{acid} and in $\Delta pH_{i\text{-}fast}$ in $AE3^{-/-}$ neurons, coupled with the faster pH_i increase in WT neurons during Cl^--free MAc, provide the first evidence that AE3 activity is a major factor in the initial phase of MAc response. The continuous pH_i drift in $AE3^{-/-}$ neurons during MAc may occur as a result of intracellular Cl^- depletion, which may decrease the activity of an acid-extruder, the Na-driven Cl/HCO3 exchanger.

Disclosures: **A.I. Salameh:** None. **W.F. Boron:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: CIHR

NSERC

MHRC

Title: Localization of equilibrative nucleoside transporter 3 (ENT3) in mouse brain

Authors: L. ROBERTS, W. XIONG, N. LEVINE, M. F. JACKSON, *F. E. PARKINSON; Univ. Manitoba, Winnipeg, MB, Canada

Abstract: Adenosine is a signaling molecule acting via cell surface G-protein coupled receptors. In brain, adenosine has neuroprotective, anticonvulsant and sedative properties; however, the mechanisms which regulate adenosine concentrations are poorly understood. Four members of the equilibrative nucleoside transporter family have been identified (ENT1-4) where ENT1 and 2 have been best characterized to date. The current study was initiated to explore the role of ENT3 in regulating brain adenosine levels. Mouse ENT3 gene sequence was inserted into pIRES puro-flag plasmid. HEK293T cells were transiently transfected then used for western blot analysis with a commercial polyclonal ENT3 specific antibody and a monoclonal antibody specific for the flag epitope. Mouse brain was dissected and mRNA was isolated from cortex, cerebellum, striatum and hippocampus. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed using ENT3 specific primers designed to amplify a 245 bp product. The ENT3-specific antibody was used in western blot analysis of proteins isolated from the dissected regions of mouse brain. Cortical astrocytes were cultured and used for RT-PCR or immunocytochemistry of ENT3. Western blot analysis performed using ENT3-transfected and wild type HEK293T cells showed a 40kD band in transfected cells only that was detected with either the ENT3 specific or the anti-flag antibody. RT-PCR analysis was positive for expression of ENT3 in all brain regions tested as well as in cortical astrocytes. Western blot analysis of dissected mouse brain regions, using the ENT3-specific antibody, showed a major band at 52kD and a minor band at 30kD. Immunocytochemistry images indicate intracellular localization of

ENT3 in astrocytes. Our results indicate that ENT3 is widely expressed in mouse brain and in cultured cortical astrocytes.

Disclosures: L. Roberts: None. **F.E. Parkinson:** None. **W. Xiong:** None. **N. Levine:** None. **M.F. Jackson:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: CRC1080

Title: Plasticity-related gene 1 specifically mediates lysophosphatidic acid uptake

Authors: *Y. LI[1], X. LIU[1], S. RICHERS[1], V. JOHANNES[1], A. MORRIS[2], J. HUAI[1], R. NITSCH[1];
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Abstract: Plasticity-related gene 1 (PRG-1, Lipid phosphate phosphatase-related protein 4 LPPR4) was firstly described by Bräuer et al. (2003) as a member of the lipid phosphate phosphatase (LPP) family. However, demonstration of robust LPA phosphatase activity associated with overexpression of this protein has been challenging (Mcdermott et al 2004). Here we report in both cellular models and primary neurons that PRG-1 can specifically bind and incorporate LPA in a nonenzymatic manner. We further defined the LPA binding pocket of PRG-1 was formed by the joint region of extracellular loop II (EL2) and transmembrane domain IV (TM4) together with the joint region of extracellular loop III (EL3) and transmembrane domain V (TM5), and the LPA uptake module of PRG-1 is composed of TM4, IL2 and TM5. Additionally the cytoplasmic tail of PRG-1 was shown to be involved in blockade of LPA uptake module at presence of LPA evoked calcium release to prevent LPA overloading. Keywords: PRG-1, LPA, LPP, uptake, transporter, calmodulin, minigene 1

Disclosures: Y. Li: None. **X. Liu:** None. **S. Richers:** None. **V. Johannes:** None. **A. Morris:** None. **J. Huai:** None. **R. Nitsch:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: EY-03592 (IAM)

NSERC DIS-0000065 (IAM)

Title: Is Inebriated-P2 an antiporter mediating transport of histamine and beta-alanine at *Drosophila* photoreceptors?

Authors: *J. BORYCZ[1], J. A. BORYCZ[1], G. UHLENBROCK[3], B. HOVEMANN[3], I. A. MEINERTZHAGEN[1,2];
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Abstract: *Drosophila* vision depends on recycling the photoreceptor neurotransmitter histamine (HA) in the first optic neuropile, or lamina. For this, the enzyme Ebony, expressed in lamina epithelial glia that surround photoreceptor terminals, conjugates β-alanine (β-Ala) to HA, to synthesise β-alanyl histamine (carcinine, CA). Concluding the recycling pathway, the enzyme Tan, expressed in photoreceptors, hydrolyses CA to release HA and β-Ala. The recycling pathway requires the cooperative action of transporters to shuttle these compounds between photoreceptor and glial cells, and these mostly have not been identified. So far only Inebriated (Ine) is proposed to act as a presynaptic transporter of CA (Gavin et al. 2007). The *inebriated (ine)* gene encodes a Na^+/Cl^--dependent SLC6 family transporter and is translated as two protein isoforms, long (P1) and short (P2). Of three *ine* mutants, ine^1 and ine^3 are null for both isoforms whereas ine^2 does not express P1 but still produces P2 (Huang et al. 2002). Photoreceptors specifically express the P2 isoform whereas P1 is expressed in non-neuronal cells. Here we report that *Drosophila* brain synaptosome preparations of *ine* mutants differentially accumulate HA, CA and β-Ala. The synaptosomes contain a heterogenous mixture of mostly neuronal elements, suggesting that fly brain synaptosomes mostly express the P2 isoform. Compared with wild-type controls the synaptosomal uptake of 0.1% CA was reduced by 52% in ine^1 and by 51% in ine^3 mutants, whereas it was increased by 90% and 84% respectively when incubated in 0.05% β-Ala. Uptake of 0.05% HA was only slightly reduced in ine^1 and ine^3. The uptake of β-Ala was ~5 fold less compared with HA or CA indicating that the capacity for ine^1 or ine^3 synaptosomes

to take up β-Ala was reduced. This difference is compatible with Ine transporting CA into photoreceptors and reciprocally transporting β-Ala back out by the same transporter, but a specific β-Ala transporter in *Drosophila* has still not been identified. The structural similarity between β-Ala and GABA suggests that both may share the same transporter (dGAT) but, declaring its ineligibility at photoreceptors, dGAT expression is restricted to glia (Martin & Krantz 2014). The presence of synaptic vesicles in histamine-deficient mutants of the synthetic enzyme *hdc* suggests that some other substance i.e. β-Ala, may be loaded into vesicles. To assess this possibility we analyzed the contents of the vesicular fraction of brain homogenates. Vesicle preparations of wild-type flies contained measurable amounts of histamine but no β-Ala, which further supports our suggestion that Ine may transport β-Ala out of the photoreceptors.

Disclosures: J. Borycz: None. **J.A. Borycz:** None. **G. Uhlenbrock:** None. **B. Hovemann:** None. **I.A. Meinertzhagen:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: FONDECYT 1140477

CMA BIOBIO (www.CMABIOBIO.cl), PIA-ECM-12

Title: Glial cell distribution and SVCT2 transporter expression in different areas of the adult brain

Authors: K. SALAZAR[1], F. MARTÍNEZ[1], F. ESPINOZA[1], *F. J. NUALART[2,1];
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Abstract: Astrocytes and specialized glial cells of the brain (i.e., tanycytes) are involved in neuroendocrine regulation, neurogenesis, blood-brain barrier formation and vitamin C recycling. To characterize the distribution and vitamin C transporter (SVCT2) expression in different regions of the brain, we have used confocal microscopy in the spectral 3D rendering mode with triple and quadruple staining. Additionally, *in situ* hybridization in combination with immunohistochemistry was performed. The expression data was confirmed using laser capture

microdissection and qPCR analysis. We used fluorescent markers for intermediate filaments (anti-GFAP and vimentin), GFP-adenovirus (ad) injected into the ventricle, and markers for glucose and vitamin C transporters (anti-GLUT1 and SVCT2, respectively). SVCT2 is not generally expressed in astrocytes; however, in the hypothalamus, alpha and beta-1 tanycytes are positive for vimentin, GLUT1 and SVCT2 (polarized to the apical membrane of the cells). Beta-2 tanycytes of the median eminence were also vimentin-, GFP-ad- and SVCT2-positive; SVCT2 was not clearly detected in GFAP-positive astrocytes of the subependymal region and median eminence. Additionally, the astrocytes detected in the external area of the entorhinal cortex and marginal zone (glia limitans) of the brain expressed SVCT2. Taken together, SVCT2 can be expressed in highly specialized astrocyte and tanycyte populations.

Disclosures: **K. Salazar:** None. **F.J. Nualart:** None. **F. Martínez:** None. **F. Espinoza:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: NIH Grant R21NSO74062A1

NIMH Grant 2P30 MH075673-06

Title: High-throughput assay development for cystine-glutamate antiporter (x_c^-)

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Abstract: The cystine-glutamate antiporter (system x_c^-) is a Na^+-independent amino acid transporter that exchanges extracellular cystine for intracellular glutamate. It is thought to play a critical role in cellular redox processes through regulation of intracellular glutathione synthesis via cystine uptake. In gliomas, system x_c^- expression is universally up-regulated while that of glutamate transporters down-regulated, leading to a progressive accumulation of extracellular

glutamate and excitotoxic cell death. Additionally, up-regulation of system x_c^- in activated microglia has been implicated in the pathogenesis of several neurodegenerative disorders mediated by excess glutamate. Consequently, system x_c^- is a potential new drug target for brain cancer and neuroinflammatory diseases associated with excess extracellular glutamate. Unfortunately no potent and selective small molecule system x_c^- inhibitors exist and to our knowledge, no high throughput screening assay has been developed to identify new scaffolds for inhibitor design. To develop such an assay, various neuronal and non-neuronal human cells were evaluated as sources of system x_c^-. Human glioma cells were chosen as the best based on xCT/*SLC7A11* expression and corresponding system x_c^- activity. Using these cells, cystine uptake and cystine-induced glutamate release assays were characterized in detail and optimized with respect to cystine and protein concentrations and time of incubation. The 384-well cystine-induced glutamate release assay was then used in a high throughput screening (HTS) campaign and the 96-well ^{14}C-cystine uptake assay was used to corroborate the results as a secondary screen following HTS. Interestingly, when comparing the rate of cystine uptake with that of glutamate release, uptake was faster than release in human glioma cells. This is in contrast to the same rates of cystine uptake and glutamate release previously reported in normal human fibroblast cells.

Disclosures: **A.G. Thomas:** None. **K. Tendyke:** None. **K.A. Loiacono:** None. **H. Hansen:** None. **V. Sahni:** None. **A. Koyama:** None. **Y. Hashizume:** None. **C. Rojas:** None. **B.S. Slusher:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.11. Glial Mechanisms

Support: CONACYT Grant 123625

Title: Regulation of the transporters involved the glutamate/glutamine cycle in cultured Müller glial cells

Authors: ***J. H. CASTILLO**[1,2], A. ORTEGA[2], A. RODRÍGUEZ[3];
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Abstract: Glutamine, the most abundant amino acid in blood, comprises one fifth of the total amino acid content. The glutamate-glutamine cycle is an example of the complex metabolic and energetic interactions between neurons and glia cells within the Central Nervous System. Through this cycle, the neurotransmitter released by glutamatergic neurons, is taken up from the synaptic cleft by glial-specific sodium dependent glutamate transporters (Excitatory amino acid transporters EAATs 1 and 2) and transformed to glutamine by the glia-enriched glutamine synthetase (GS). The reverse-mode of operation of glial sodium-dependent neutral amino acid transporters (SNATs 3 and/or 5) releases glutamine into the extracellular space, to be finally taken up by neurons by System A glutamine transporters (SNAT1 and/or 2). Despite of the fact that this cycle was originally described more than twenty years ago, the molecular events that regulate not only its activity, but also the expression of its components begins now to be elucidated. Using cultured Müller glia cells we report here the characterization of [^{3}H] L-glutamine uptake activity and its regulation by glutamate. Furthermore, we provide evidence for the functional expression of EAAT1 and SNAT3 in these retinal radial glia cells. Moreover, preliminary data suggests that a functional and physical coupling between EAAT1 and SNAT3 in present in Müller glia and that this biochemical interaction is dependent upon glutamate stimulation. These results favor the notion of a critical role of SNATs in glutamatergic neurotransmission.

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Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: IVIC151

Title: Effect of zinc deficiency on taurine concentration, localization of taurine and zinc transporter, taurine tranport and RNAm levels taurine transporter in cells rat retina

Authors: A. MARQUEZ GARCIA[1], M. URBINA[1], V. SALAZAR[2], *L. LIMA[1];
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Abstract: Taurine and zinc are highly concentrated in the retina, they interact to modify photoreceptor plasma membranes, are antioxidants and have trophic effects, affecting

morphology and function of the retina. There is limited evidence about the effects of zinc on taurine system in mammalian retina. The objectives of the present study are to evaluate the effect of zinc deficiency on taurine concentration, taurine transport and taurine transporter (TAUT) mRNA in cells of rat retina. The intracellular chelator of zinc, N,N,N,N-tetrakis-(2-pyridylmethyl) ethylenediamine (TPEN) was used *in vivo* to determine levels of zinc by spectrophotometry (ICP), taurine concentration by HPLC, localization of TAUT and ZnT in retina by immunohistochemistry, taurine[3H] transport and relative levels of TAUT mRNA by RT-PCR. TPEN, was injected io for 10 days, at concentrations of 1, 2.5 and 5 nM. Zinc decreased at 5 days with 5 nM TPEN. Taurine concentration significantly diminished by 66.62% 5 days after io administration of 5 nM TPEN. TAUT was localized in all retinal layers, with significantly intense staining in ganglion cell layer (GC), inner and outer nuclear layers (INL and ONL) and photoreceptors (Phot) than in inner and outer plexiform layers (IPL and OPL). ZnT-1, 3 and 7 had intense staining in the GC, IPL, OPL and Phot. After 5 days of treatment with 5 nM TPEN, localization of TAUT and ZnTs significantly decreased in retinal layers. Taurine[3H] transport was standardized for incubation time, temperature and Na+ dependence, cell number, effect of β-alanine and hypotaurine and saturation assays. The experimental conditions were: 25 seconds at 37 °C and 200,000 cells per tube. β-Alanine and hypotaurine decreased transport. Saturation assays showed the existence of two components. After 5 days of treatment with 5 nM TPEN, taurine[3H] transport significantly decreased. The relative levels of TAUT mRNA decreased after 5 days of treatment with 5 nM TPEN. The results indicate that zinc is required for taurine system in the retina, which involves taurine levels, operation of taurine transport and TAUT mRNA levels. All these evidences make to suggest that zinc and taurine interact at different levels in the retina, reinforced a crucial interplay of both molecules.

Disclosures: **A. Marquez Garcia:** None. **M. Urbina:** None. **L. Lima:** None. **V. Salazar:** None.

Poster

687. Acetylcholine and Other Transporters

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Topic: B.05. Transporters

Support: Foundation P. E. Kempkes No. 01/2012

Title: Comparative analysis of VNUT(SLC17a9) mRNA expression in neurons and neuroglia in mouse brain

Authors: *M. K.-H. SCHAFER, S. DOTZAUER, P. REINOSS, M. A. R. BERTOUNE; Mol. Neurosci., Philipps Univ. Marburg, Marburg, Germany

Abstract: ATP and other nucleotides have long been recognized as important neurotransmitters in the so-called purinergic neurotransmission.They are often co-released with classical neurotransmitters from synaptic vesicles to modulate postsynaptic signaling in neuronal networks and in neuroglial transmission as well. The solute carrier protein SLC17A9 was identified as a vesicular transporter for ATP and other nucleotides (VNUT) by Sawada et al. in 2008. Since then VNUT expression has been reported in the CNS and PNS in neurons and neuroglia and in peripheral tissues such as endocrine and exocrine glands and in immune cells. While its role in ATP release from microglia has recently been demonstrated, the extent of VNUT expression and its role in astrocytes is less clear. The aim of this study was to characterize the cellular gene expression of VNUT in neurons and neuroglia in the adult mouse brain in comparison to peripheral tissues using *in situ* hybridization, laser-microdissection assisted RT-PCR analysis and qPCR. Using RT-qPCR VNUT mRNA could be measured in extracts of many brain regions. Surprisingly, VNUT mRNA levels in spleen, primary microglial culture and the cell line BV2 were between 10 to 100 fold higher than in neuronal extracts of any brain region. In addition, VNUT RNA transcripts were also more than 10 fold higher in cultured microglia than in primary astrocyte cultures. The apparently low neuronal VNUT expression was confirmed by RT-PCR analysis of laser-microdissected neurons from neocortex and hippocampus. Using ISH with highly sensitive riboprobes directed against different regions of the mouse VNUT mRNA we could not detect specific neuronal hybridization signals in mouse brain including hippocampus, although all VNUT probes used produced robust signals in peripheral tissues such as exocrine glands. Our results of high VNUT transcript levels in microglial cultures are in support of recent evidence suggesting a VNUT-dependent exocytotic mechanism of ATP release from microglia. The very low levels of VNUT mRNA in neurons and astroglia compared to that of other neurotransmitter transporters raises questions of the importance of VNUT dependent storage and release of nucleotides from neurons or astroglia for purinergic signaling in many brain regions. Supported by Foundation P.E. Kempkes No. 1/2012 (To M.A.R.B.)

Disclosures: **M.K. Schafer:** None. **S. Dotzauer:** None. **P. Reinoss:** None. **M.A.R. Bertoune:** None.

Poster

687. Acetylcholine and Other Transporters

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 687.19/B22

Topic: B.05. Transporters

Title: The environmental neurotoxin β-N-methylamino-L-alanine: Uptake and effects in sh-sy5y and u343 cells and intracellular fibril formation in hippocampus following neonatal exposure

Authors: *L. ERSSON[1], M. ANDERSSON[2], A.-L. BERG[3], J. HANREIDER[4,5], O. KARLSSON[1], E. B. BRITTEBO[1];
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Abstract: The environmental neurotoxin β-N-methylamino-L-alanine (BMAA) has been implicated in the etiology of neurodegenerative disease. BMAA is a developmental neurotoxicant that can induce long-term learning and memory deficits, as well as regionally restricted neuronal degeneration in rats. The present studies aim to characterize 1) the uptake of ^{14}C-BMAA in the human neuroblastoma cell line SH-SY5Y and the human glioblastoma cell line U343 and 2) the long-term changes in the hippocampus of adult rats treated neonatally (postnatal days 9-10) with BMAA (460 mg/kg) using transmission electron microscopy and laser capture microdissection followed by LC-MS/MS for proteomic analysis. There was a rapid uptake of BMAA in both cell lines. Competition experiments with other amino acids (under normal and sodium free conditions) indicated that BMAA may be taken up by other transporters than the large amino acid transporters 1 and 2 (LAT 1 and LAT 2) in these cells. The ultrastructural examination of the hippocampus from the *in vivo* experiment revealed intracellular deposition of abundant bundles of closely packed parallel fibrils in neurons, axons and astrocytes of the hippocampus CA1 in BMAA-treated rats. Proteomic analysis of hippocampus CA1 demonstrated an enrichment of chaperones, cytoskeletal and intermediate filament proteins and proteins involved in the antioxidant defense system. The present studies demonstrate that BMAA-treatment of SH-SY5Y/U343 cells and neonatal exposure to BMAA will be useful models for further *in vitro* and *in vivo* studies on BMAA-induced fibril formation.

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Poster

688. Homeostatic Synaptic Plasticity and Presynaptic Function

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Topic: B.08. Synaptic Plasticity

Title: The role of type VI adenylyl cyclase in the regulation of synaptic activity

Authors: *C.-P. CHANG[1,3], C.-T. LEE[4], M.-S. LIN[1], H.-L. LAI[1], C.-L. CHIEN[1], P.-L. CHENG[2], C.-C. LIEN[4], Y. CHERN[1];
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Abstract: Type VI adenylyl cyclase (AC6) is a membrane bound, calcium-regulated adenylyl cyclase which mediates the synthesis of cAMP from ATP during extracellular stimulation. Unlike other ACs, AC6 is of particular interest in the brain as it is expressed in neuronal cells and distributed in various brain regions. Furthermore, it is negatively regulated by multiple signals (such as Gαi, Ca2+, PKA, PKC, and NO). Therefore, AC6 might serve as a key integrator in maintaining proper neuronal functions. In addition to the production of cAMP, we previously showed that the N-terminus of AC6 modulates neurite outgrowth by interacting with Snapin in primary hippocampal neurons and Neuro2A cells, which revealed that AC6 is an important modulator of neuritogenesis. Nonetheless, the physiological functions of AC6 in the central nervous system remain elusive. By using AC6 KO mice, we found that genetic removal of AC6 increased dendritic spine density of hippocampal CA1 pyramidal neurons without affecting the gross anatomy of the brain. Besides, electrophysiological analyses showed increased membrane time constant, lower rheobase, reduced action potential threshold, and an increase in the ratio of NMDA to AMPA receptor-mediated EPSCs in CA1 pyramidal neurons of AC6 KO mice, indicating that AC6 negatively modulates neuronal excitability. Furthermore, the glutamate-induced cAMP/calcium responses were enhanced in primary hippocampal neurons of AC6 KO mice. Our results demonstrated that AC6 plays pivotal roles in regulating in the glutamate-dependent neuronal activity in the hippocampus.

Disclosures: C. Chang: None. **C. Lee:** None. **M. Lin:** None. **H. Lai:** None. **C. Chien:** None. **P. Cheng:** None. **C. Lien:** None. **Y. Chern:** None.

Poster

688. Homeostatic Synaptic Plasticity and Presynaptic Function

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Title: Unveiling the puzzle of homeostatic plasticity | How Contactin-associated proteins 1 and 2 (Caspr1 & 2) fit in to homeostatically regulate synaptic AMPA receptors

Authors: *D. FERNANDES[1,2,3], L. RIBEIRO[1], S. SANTOS[1], A. CARVALHO[1,4];
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Abstract: Consolidation of our memories and the translation of our experience into learning rely on changes in synaptic function through several forms of synaptic plasticity including Hebbian and homeostatic plasticity. In turn, the expression of these plastic events is highly dependent on the regulation of synaptic AMPA receptors (AMPARs), through mechanisms still poorly understood. Moreover, it is thought that there is an intricate puzzle of yet undiscovered interacting proteins that may contribute to the regulation of AMPA receptors and be crucial for plasticity events. In this context, our lab has recently shown that the cell-adhesion molecule Contactin-associated protein 1 (Caspr1) is a novel AMPAR interactor, able to regulate the basal trafficking of the GluA1 subunit into synapses. We now found evidence for an additional posttranscriptional role for Caspr1 in increasing GluA1 mRNA and regulating the phosphorylation of the RNA-binding protein Zipcode-binding protein 1 (ZBP1). Our results indicate that ZBP1 binds to GluA1 mRNA, an interaction that significantly decreases upon chronic changes in neuronal activity. Indeed, we show that chronic blockade of activity

significantly upregulates not only total mRNA levels and surface synaptic puncta of GluA1, but also phosphorylation levels of ZBP1. Additionally, we demonstrate that Caspr1 expression is regulated by neuronal activity, since its total protein and mRNA levels increase upon homeostatic stimuli. Caspr2, another Contactin-associated protein already implicated in autism-spectrum disorders and identified as an antigen in autoimmune encephalitis, also interacts with the GluA1 subunit. Furthermore, we show that synaptic puncta of Caspr2 significantly increase upon chronic blockade of activity, suggesting a possible function for Caspr2, in parallel with Caspr1, in regulating homeostatic plasticity. Indeed, when we knock-down the endogenous expression of either Caspr1 or Caspr2, not only does the basal synaptic content of GluA1-containg AMPARs decrease, but also the GluA1 synaptic upscaling induced by chronic blockade of activity is blocked. Altogether, our results identify Contactin-associated proteins as two major novel regulators of synaptic AMPA receptors and suggest a crucial role for these proteins in the expression of homeostatic plasticity phenomena.

Disclosures: **D. Fernandes:** None. **L. Ribeiro:** None. **S. Santos:** None. **A. Carvalho:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant T32GM008361

NIH Grant 5T32NS061788-05

Title: Epigenetic regulation of homeostatic synaptic scaling via DNA methylation

Authors: ***J. MEADOWS**[1], M. GUZMAN KARLSSON[2], C. HOLLEMAN[2], J. DAY[2], J. HABLITZ[2], D. SWEATT[2];
[1]Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Activity-dependent alterations in synaptic strength represent a key cellular mechanism of learning and memory. Synapse-specific plasticity, including Hebbian long-term potentiation (LTP) and long-term depression (LTD), has long been thought to play a major role in information storage. However, both LTP and LTD can propagate in a feed-forward manner,

disrupting synaptic gain and network equilibrium. Thus neuronal networks utilize homeostatic synaptic plasticity (HSP) to counterbalance these synapse-specific changes. Synaptic scaling, a type of HSP, is characterized by cell-wide modifications of postsynaptic receptor density occuring in response to chronically elevated or depressed neuronal activity. Importantly, during synaptic scaling, changes in postsynaptic receptor density are thought to occur multiplicatively, preserving relative synaptic strengths acquired via synapse-specific plasticity. Mechanisms that regulate synaptic scaling are incompletely understood. However, emerging evidence suggests that synaptic scaling depends on transcriptional and epigenetic regulation. One type of epigenetic mechanism, DNA methylation, has been reliably shown to be important for learning and memory. We hypothesized that synaptic scaling is dependent on DNA methylation. In studies using dissociated cultures of cortical neurons, we have confirmed that synaptic scaling of mEPSCs occurs in a bi-directional, multiplicative manner in response to chronic changes in activity. We then examined molecular changes underlying synaptic scaling and found that chronically changed neuronal activity regulates the expression of enzymes involved in active DNA methylation and de-methylation. Furthermore, the small-molecule, competitive DNA methyltransferase inhibitor, RG108, blocks both the scaling up and down of mEPSCs, demonstrating that bi-directional scaling is dependent upon active DNA methylation. Together, our results suggest a critical role for DNA methylation in homeostatic synaptic scaling.

Disclosures: J. Meadows: None. **J. Hablitz:** None. **M. Guzman Karlsson:** None. **J. Day:** None. **D. Sweatt:** None. **C. Holleman:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: PKA-GluA1 coupling via AKAP5 controls AMPA receptor phosphorylation and cell-surface targeting during bidirectional homeostatic plasticity

Authors: *G. H. DIERING[1], A. GUSTINA[2], R. HUGANIR[2];
[1]Neurosci., [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Bidirectional synaptic plasticity occurs locally at individual synapses during LTP or LTD, or globally during homeostatic scaling. LTP, LTD, and homeostatic scaling alter synaptic strength through changes in post-synaptic AMPARs, suggesting the existence of overlapping

molecular mechanisms. Phosphorylation is critical for controlling AMPAR trafficking during LTP/LTD. Here we addressed the role of AMPAR phosphorylation during homeostatic scaling. We observed bidirectional changes in PKA phosphorylation of GluA1 S845, during scaling, resulting from a loss of PKA from the synapse during scaling-down and enhanced activity of PKA in the synapse during scaling-up. Altered synaptic PKA signaling, requiring the scaffold AKAP5, alters the effectiveness of neuromodulators and NMDAR activation. Increased phosphorylation of S845 drove scaling-up while mutation of S845 blocked scaling-up. Finally we show that AMPARs scale differentially based on their phosphorylation status at S845. These results show that rearrangement in PKA signaling controls AMPAR phosphorylation and surface targeting during homeostatic plasticity.

Disclosures: G.H. Diering: None. **R. Huganir:** None. **A. Gustina:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NSF GRFP fellowship 401925

Title: Synapse type specificity of synaptic scaling in visual cortical L4

Authors: *N. J. MISKA, G. G. TURRIGIANO;
Neurosci., Brandeis Univ., Waltham, MA

Abstract: Homeostatic synaptic scaling contributes to the stabilization of neuronal firing *in vivo* following experience-dependent perturbations, and is thought to involve global changes in all excitatory postsynaptic strengths onto a neuron. However, there is recent *in vitro* evidence for the existence of local, synapse-specific forms of homeostatic synaptic plasticity that are mechanistically distinct from synaptic scaling. Whether these local forms of homeostatic synaptic plasticity operate *in vivo* has not been established. Here we developed a system for probing changes in quantal amplitude at targeted synaptic subsets, and we are using this system to determine whether convergent synaptic subsets are regulated differentially during experience-dependent homeostatic plasticity. We optogenetically evoke desynchronized vesicle release selectively from thalamocortical (TC) synapses or from lamina-specific intracortical (IC) synapses onto L4 star pyramidal neurons in acutely prepared brain slices of primary visual cortex

(V1). Previously, our lab found that monocular deprivation (MD) first reduces firing in V1 after 2d, but that firing rebounds to baseline over the next several days. Consistent with this, we show that 2d of MD significantly depresses evoked TC quantal amplitude compared to sham deprived animals (83.86±4.89% of control, $P < 0.05$, one-way ANOVA followed by Tukey-Kramer posthoc test). This initial depression is followed by a potentiation after 4 days of MD compared to the sham condition (difference in potentiation between MD and control = 124.25±6.40%), consistent with the induction of homeostatic synaptic plasticity at TC synapses. Future work will compare changes in quantal properties at TC synapses with those evoked from L4 IC synapses to determine whether this homeostatic potentiation occurs globally across synapse types or is locally induced at TC synapses.

Disclosures: **N.J. Miska:** None. **G.G. Turrigiano:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH R01-EY014882

Title: Experience-dependent synaptic scaling requires metabotropic glutamate receptor signaling in mouse primary visual cortex

Authors: ***H.-K. LEE**[1], V. CHOKSHI[2], P. WORLEY[3], M. GAO[4];
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Abstract: Visual experience modulates the synaptic strength of cortical neurons in mouse primary visual cortex. When the animals are dark reared for two days during their critical period, the synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mediated miniature excitatory postsynaptic current (mEPSC) amplitude increases in L2/3 of visual cortex. Furthermore, when these dark reared animals are exposed to just two hours of light, the AMPA receptor mEPSC amplitude decreases back to normal levels. We hypothesize that metabotropic glutamate receptors, mGluR5, presented perisynaptically act as activity sensors to modulate synaptic strength with increased synaptic activity. These receptors are coupled to long forms of scaffolding protein, Homer1. An alternative splicing product of Homer1 protein, Homer1a, is

induced upon neuronal activity, and it decouples mGluR5 from the long forms of Homer1. When the AMPA receptor currents were measured in transgenic animals with abolished Homer1a expression or binding to mGluR5, we found that this experience-dependent scaling is abolished and mEPSC amplitude remains large as observed in dark reared wild type animals. Our results suggest that mGluR5 signaling is required to maintain experience-dependent homeostatic scaling of synaptic strength.

Disclosures: H. Lee: None. **V. Chokshi:** None. **M. Gao:** None. **P. Worley:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH R01 NS039313 to GWD

Title: Cdk5 is necessary for the rapid induction of presynaptic homeostasis

Authors: *C. J. LOCKE, K. J. FORD, G. W. DAVIS;
Dept. of Biochem. & Biophysics, Univ. of California, San Francisco, San Francisco, CA

Abstract: In response to decreased postsynaptic neurotransmitter receptor sensitivity, the neuromuscular junction (NMJ) homeostatically potentiates neurotransmitter release to maintain a set point level of activity. This form of homeostatic plasticity is evolutionarily conserved from *Drosophila* to humans. Currently, the molecular mechanisms underlying this robust and accurate form of homeostatic plasticity are not well understood. Through an ongoing electrophysiology-based genetic screen in *Drosophila*, we have uncovered a key role for cyclin-dependent kinase 5 (Cdk5) in synaptic homeostasis. We determined that molecular null mutations in *cdk5* and *p35*, which encodes a critical activator of Cdk5, completely disrupt the rapid homeostatic potentiation of glutamate release induced by philanthotoxin-433 (PhTx) treatment at the fly NMJ. Using two-electrode voltage-clamp, we attribute this failure of short-term homeostatic plasticity to an impairment in Cdk5-dependent modulation of the readily-releasable vesicle pool size. In addition, spatiotemporally controlled expression of a dominant-negative Cdk5 by the TARGET system demonstrated that Cdk5-mediated phosphorylation is required acutely in the presynaptic motor neuron for the rapid induction of synaptic homeostasis. Conversely, we discovered that Cdk5-deficient flies exhibit a robust increase in glutamate release following chronic reduction of

glutamate sensitivity, sustained through the genetic ablation of GluRIIA. These results suggest that the mechanisms underlying short-term and long-term forms of homeostatic plasticity are distinguishable by Cdk5 activity.

Disclosures: **C.J. Locke:** None. **G.W. Davis:** None. **K.J. Ford:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: National Institute on Aging Intramural Research Program, NIH

Title: Involvement of sirt3 in homeostatic synaptic plasticity

Authors: ***Y. LIU**, A. CHENG, D. LU, M. MATTSON;
NIA, NIH, Baltimore, MD

Abstract: By generating ATP and regulating local Ca2+ dynamics, mitochondria influence synaptic plasticity. The mitochondrial histone deacetylase Sirt3 is believed to bolster mitochondrial health, but its possible roles in synaptic plasticity are unknown. We therefore evaluated synaptic transmission and plasticity in cultured hippocampal neurons from embryonic wild type (WT) and Sirt3 knockout (KO) mice, with a focus on synaptic scaling, a type of homeostatic plasticity that allows neurons to regulate their firing rate. We found that the GABAa receptor blocker picrotoxin (1 micromolar) rapidly increased neuronal firing rate and increased reactive oxygen species (ROS) levels, and also increased expression of Sirt3 measured at 6 and 48 hours. Picrotoxin treatment decreased the amplitude of mEPSC (miniature excitatory postsynaptic currents) in WT neurons within 24-48 hours, but failed to down-regulate EPSCs in Sirt3 KO neurons. The level of ROS returned to baseline in WT neurons within 24-48 hours of exposure to picrotoxin, whereas ROS levels remained elevated in Sirt3 KO neurons. Exposure of WT neurons to 5 micromolar hydrogen peroxide prevented picrotoxin-induced synaptic down-scaling. Our findings reveal a role for Sirt3 in homeostatic synaptic scaling, and suggest that Sirt3 may be particularly important in preserving this form of synaptic plasticity in conditions where neurons experience hyperactivity and oxidative stress.

Disclosures: **Y. Liu:** A. Employment/Salary (full or part-time):; NIA, NIH. **A. cheng:** None. **M. Mattson:** None. **D. Lu:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: CIHR

NSERC

FRQS

Title: The role of dystroglycan and agrin in mediating homeostatic plasticity at GABAergic synapses

Authors: ***H. PRIBIAG**[1,2], H. PENG[2], W. A. SHAH[2], S. CARBONETTO[2], D. STELLWAGEN[2];
[1]Montreal Gen. Hosp., Montreal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Mechanisms of homeostatic plasticity operating at GABAergic synapses remain poorly understood. Factors that may regulate the abundance of GABA(A)Rs at inhibitory synapses in response to chronic changes in neuronal activity are attractive candidates for the regulation of inhibitory homeostatic plasticity. Dystroglycan (DG), a cell adhesion molecule well known to be essential for skeletal muscle integrity and the formation of neuromuscular synapses, is also localized post-synaptically at GABAergic synapses in the CNS. Mutations that affect DG function not only result in muscular dystrophies, but also in severe cognitive deficits and epilepsy. Here we investigate the role of DG in homeostatic plasticity of GABAergic synaptic strength during prolonged elevation of neuronal activity induced by bicuculline treatment. Treatment with bicuculline for 24 hours increases surface and total DG expression as well as clustering of DG colocalized with GABA(A)Rs. Inhibition of protein synthesis prevents this activity-dependent accumulation of synaptic DG and GABA(A)Rs, and blocks scaling up of inhibitory neurotransmission. Critically, RNAi-mediated knockdown of DG also blocks scaling up of inhibitory synapses, as does knockdown of LARGE - a glycosyltransferase essential for DG function. The DG ligand agrin increases GABA(A)R clustering and mimics bicuculline-induced inhibitory scaling up in a DG-dependent manner, indicating that activation of this pathway alone is sufficient to regulate GABA(A)R trafficking. We are further investigating

whether activity-dependent agrin cleavage mediates this plasticity, and are identifying other essential signaling components in this pathway. These data demonstrate that DG and LARGE-dependent glycosylation are important factors for homeostatic synaptic plasticity at inhibitory synapses.

Disclosures: **H. Pribiag:** None. **H. Peng:** None. **W.A. Shah:** None. **S. Carbonetto:** None. **D. Stellwagen:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS035812

Title: CaMKII regulates kinesin-mediated AMPAR transport and homeostatic control of synaptic strength

Authors: ***F. J. HOERNDLI**, J. E. MELLEM, R. WANG, A. KALLARACKAL, P. J. BROCKIE, C. THACKER, D. M. MADSEN, A. V. MARICQ;
Biol., Univ. of Utah, SALT LAKE CTY, UT

Abstract: Synaptic transmission is critically dependent on processes that maintain an optimal number of postsynaptic neurotransmitter receptors. Transmission at most excitatory synapses is mediated by the AMPA subtype of ionotropic glutamate receptors (AMPARs). In *C. elegans*, the microtubule-dependent motor kinesin-1 maintains synaptic strength by delivering and removing synaptic AMPARs. Here, we show that UNC-43, the sole *C. elegans* homolog of Ca^{2+}/calmodulin dependent kinase CaMKII, functions in a cell autonomous fashion to regulate motor-driven transport of AMPARs and thus synaptic strength. In loss-of-function *unc-43* mutants, bidirectional transport of GLR-1 AMPARs is greatly decreased, as are the rates of delivery and removal of synaptic GLR-1. Acute, spatially restricted ablation of UNC-43/CaMKII revealed that it has essential roles in both the loading of AMPAR cargo onto motors and in the membrane insertion of AMPARs. Consistent with these functions, we found that the amplitude of glutamate-gated current was greatly decreased in *unc-43* mutants. In wild-type worms, synaptic strength was maintained at a constant level even if challenged by perturbations such as the overexpression of the AMPAR signaling complex. In dramatic contrast, glutamate-gated

currents were greatly increased in *unc-43* mutants that overexpressed the complex. Remarkably, defects in both GLR-1 transport and glutamate-gated current in *unc-43* mutants were rescued in transgenic worms that expressed mammalian CaMKII. Our results demonstrate that UNC-43/CaMKII has an unanticipated role in regulating AMPAR transport and is essential for the homeostatic maintenance of synaptic strength. By regulating the motor-driven delivery and removal of synaptic AMPARs, UNC-43/CaMKII rapidly establishes receptor number at proximal and distal synapses, thus maintaining neuronal information processing.

Disclosures: **F.J. Hoerndli:** None. **J.E. Mellem:** None. **R. Wang:** None. **A. Kallarackal:** None. **P.J. Brockie:** None. **C. Thacker:** None. **D.M. Madsen:** None. **A.V. Maricq:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS062738

Title: Synaptic homeostasis is a reversible process at the *Drosophila* neuromuscular junction

Authors: *C. J. NEFF[1], C. A. FRANK[2];
[1]Interdisciplinary Grad. Program in Neurosci., [2]Dept. of Anat. and Cell Biol., Univ. of Iowa, Iowa City, IA

Abstract: The ability of a synapse to maintain a physiologically appropriate output is necessary for all modalities of neurological function. Sensory, motor, and behavioral systems across metazoan nervous systems depend on faithful circuit output and homeostatic mechanisms to maintain this output. Failure of these systems to regulate their output could contribute to a number of disease states including ataxia, migraine, and epilepsy. However, aspects of homeostatic plasticity are still poorly understood. In this study, we have addressed whether induction of a signaling pathway that leads to homeostatic compensation is a reversible process. The *Drosophila* neuromuscular junction (NMJ) is an excellent system to study synaptic homeostasis. Impairment of muscle sensitivity to glutamate, using either a genetic or pharmacological challenge, induces a retrograde muscle-to-nerve signal that produces a corresponding increase in the amount of presynaptic glutamate released during evoked potentials. As a result, levels of muscle excitation are homeostatically maintained. For our

research, we are employing *Drosophila* genetic tools to allow us to modulate the time course on which the system undergoes a homeostatic challenge. We provide an initial challenge and subsequently remove it, allowing us to determine whether the neurotransmitter release returns to wild-type levels after the challenge is removed. We have found that homeostatic upregulation of vesicle release is reversed after removal of a challenge, such as postsynaptic expression of a dominant negative glutamate receptor subunit. We have also examined homeostatic downregulation of vesicle release. Presynaptic overexpression of *Drosophila* vesicular glutamate transporter (VGlut) leads to a decrease in the amount of glutamate released during evoked potentials. Our data suggest that the signaling processes involved in both homeostatic upregulation and downregulation are reversible, and in absence of a challenge, vesicle release levels return to that of an unchallenged NMJ. Finally, we are using genetic approaches to characterize what molecules are necessary for the reversibility of synaptic homeostasis; progress on these efforts will be reported.

Disclosures: C.J. Neff: None. **C.A. Frank:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH R01-EY014882 to H-K Lee

Title: Role of NMDA receptors in experience-dependent homeostatic synaptic scaling

Authors: G. RODRIGUEZ[1], *J. L. WHITT[1], M. GAO[2], H.-K. LEE[1];
[1]Zanvyl Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD; [2]Div. of Neurol., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Loss of visual experience can lead to dramatic changes of synaptic connections in the visual cortex (V1). Many of the changes observed are thought to be the result of homeostatic mechanisms taking place as a result of visual deprivation. It has previously been demonstrated that AMPA receptor mediated miniature excitatory post synaptic currents (mEPSCs), in layer 2/3 of the visual cortex, show larger amplitudes after visual deprivation for two days. These results indicate that the cortex strengthens excitatory synapses after loss of activity allowing it to reach its target firing rate. Moreover it was shown that reexposure to light for two hours reverses these

effects, decreasing AMPAR mEPSCs amplitudes back to baseline levels. However, it is still not clear whether NMDA receptor activation contributes to homeostatic plasticity *in vivo*. In this study we take advantage of an NR1 flox mouse line to molecularly knock out NMDA receptors in layer 2/3 of the visual cortex with *in vivo* viral injections of a Cre-GFP adenovirus. Injected mice were either normal reared for 3 weeks, visually deprived in a dark room for 2 days after 3 weeks of normal vision or visually deprived and reexposed to light for two hours. Age matched non injected mice or wildtype mice injected with Cre-GFP were subjected to the same conditions and used as controls. AMPARs mEPSCs were recorded from all groups and compared. We show that NMDARs are necessary for experience dependent synaptic scaling. **Support:** NIH R01-EY014882 to H-K Lee.

Disclosures: **G. Rodriguez:** None. **J.L. Whitt:** None. **M. Gao:** None. **H. Lee:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: R01-EY022720

F31-NS079058

Title: Cross-modally induced changes in auditory cortex circuitry after visual deprivation

Authors: *E. R. PETRUS, H.-K. LEE;
Johns Hopkins Univ., Baltimore, MD

Abstract: The loss of one sensory modality can trigger compensatory changes in the spared senses (Bavelier and Neville, 2002). The mechanism underlying this compensation was first observed at the synaptic level by scaling down of miniature excitatory post synaptic currents (mEPSCs) in primary auditory (A1) and somatosensory (S1) cortices following the loss of vision (Goel et al., 2006), which occurs in opposite polarity to those observed in V1 (Desai et al., 2002). These homeostatic adaptations to the loss of vision observed in A1 are thought to be in response to increased auditory signal arriving to A1, which arrives via potentiation of thalamocortical (TC) inputs (Petrus et al., 2014). Stronger auditory signal is then amplified in the thalamo-recipient layer 4 (L4) of A1 before being passed to superficial layers 2/3 (L2/3). These

changes are accompanied by alterations in spontaneous and evoked inhibitory transmission which are also recruited by cross-modal sensory loss. Inhibition is regulated in a lamina dependent, age independent manner, such that evoked inhibition from parvalbumin expressing (PV+) neurons is stronger to L4 neurons in A1, but miniature inhibitory post synaptic currents (mIPSCs) increase in frequency to L2/3 pyramidal neurons. Together these changes demonstrate cross-modal plasticity is able to create both widespread homeostatic adaptations in spared sensory cortex, but also targeted, synapse specific alterations to more finely tune the spared auditory cortex after loss of vision.

Disclosures: E.R. Petrus: None. **H. Lee:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: Homeostatic plasticity in the adult mouse visual cortex (V1)

Authors: M. TEICHERT, W. KARGUTH, A. DÖDING, K. LEHMANN, *J. BOLZ;
Univ. Jena, Jena, Germany

Abstract: It has been suggested that homeostatic plasticity maintains neuronal activity around a stable point (Turrigano et al., 1998). However, most previous work has been performed *ex vivo* and so far there is only one study which examined homeostatic plasticity in the adult visual cortex *in vivo* (Keck et al., 2013). These authors showed that after bilateral retinal lesions there is first a decrease in firing rates in V1 followed by a gradual increase back to control levels. Obviously, the repercussions of homeostatic mechanisms on visual function and related behaviors can not be studied after retinal lesions. We therefore used a different approach to decrease activity levels in adult V1. For this we engineered fibroblasts to produce GAD65 and the reporter mCherry. We then transplanted these cells at two sites flanking the binocular region of V1 in mice 110 days or older. We first verified that these cells produce and release GABA for several weeks. We used a novel technique for non-invasive chronic optical imaging (Teichert et al., 2014) and performed recordings every other day starting the day after engrafting for the next 9 days. Results show that one day after transplantation activity in V1 was unchanged. However, 3 days after transplantation activity levels in almost the whole binocular region (-5° to + 15° azimuth; -17° to + 41° elevation) were significantly reduced. Then, after another 2 days,

neuronal activity was back to baseline levels. No alterations in firing levels were observed in control experiments with transplanted mCherry fibroblasts without the GAD65 construct. These data indicate that due to tonic GABAergic inhibition there is a homeostatic up-regulation that stabilizes firing rates in adult V1. Currently we are examining the functional consequences before and after homeostasis *in vivo*.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH NINDS NRSA 1 F32 NS078859-01A1

Title: Homeostasis of cortical neuronal activity is differentially expressed during waking and sleeping in freely behaving animals

Authors: ***K. B. HENGEN**, S. D. VAN HOOSER, A. J. KASHANCHI, G. G. TURRIGIANO; Biol., Brandeis Univ., Waltham, MA

Abstract: It is theorized that much of the neurological benefit conferred by sleep arises from the state-dependent expression of synaptic homeostasis. However, the role of sleep in shaping and regulating neuronal activity at the level of individual neurons is not understood. A significant barrier to progress is our nearly complete lack of insight into homeostatic plasticity in the intact brain. We previously demonstrated that cortical neuronal activity is regulated homeostatically in the freely behaving animal, independent of sleep state, around a firing rate set point. These data indicate that the firing rate set point of neurons supersedes state-dependent changes in neuronal activity. To investigate the role of sleep in the regulation of neuronal activity, we recorded activity from ensembles of cortical single units continuously for 10 days during a monocular deprivation (MD) paradigm. We then examined the homeostatic rebound of spontaneous activity during prolonged MD as a function of neuromodulatory state. Our preliminary data suggest that functional homeostatic plasticity in the neocortex emerges during bouts of waking and not sleep. Further, our data reveal circadian oscillations in the spontaneous activity patterns of individual neurons in the primary visual cortex independent of MD. These data offer novel insights into the

necessity of environmental cues such as light/dark transitions as well as behavioral factors such as sleep cycles to the proper regulation of neuronal activity.

Disclosures: K.B. Hengen: None. **S.D. Van Hooser:** None. **A.J. Kashanchi:** None. **G.G. Turrigiano:** None.

Poster

688. Homeostatic Synaptic Plasticity and Presynaptic Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: CIHR

NSERC

ALS Canada

Title: Neuronal activity regulates axonal growth during motor neuron development *in vitro*

Authors: *E. K. AUBREY, V. F. RAFUSE;
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Abstract: Propagating waves of spontaneous, highly rhythmic electrical activity occurs along the neuroaxis of the developing spinal cord when motor axons are making their initial pathfinding decisions as they exit the neural tube. While the precise function of this rhythmic activity is not known, previous in ovo studies indicate that it is involved in motor axon pathfinding. This assumption is based on studies where motor axons were shown to grow to inappropriate targets when rhythmic activity was modestly increased or decreased. Furthermore, experimental reduction in rhythmic activity in ovo changed the level of expression of several motor axon guidance molecules. While it is clear that the pattern of rhythmic activity influences motor axon pathfinding during early neuromuscular development, the cellular mechanisms by which it does so remains poorly understood. Here we developed an *in vitro* model system where motor neuron activity can be precisely regulated via light-gated ion channels. More specifically, mouse motor neurons were derived from embryonic stem (ES) cells expressing channelrhodopsin2 and eGFP under a motor neuron specific promoter (Hb9). ES cell-derived motor neurons were then cultured for 48 hours in complete darkness, or were activated with blue light emitting diodes for 100 ms every 10, 40 or 75 seconds. In some studies, tetrodotoxin (TTX)

was added to the cultures to block action potentials. Overall neurite length (nl), longest distance traveled by a single neurite (dt), and the number of neurite branches (bn) were quantified. To date, nl and bn were found to be significantly higher in light activated cultures compared to those grown in the dark, irrespective of the pattern of activation. In contrast, dt was only higher in cultures stimulated with light every 40 seconds. All three measures were the same in cultures treated with TTX, regardless of whether they were activated with light or grown in the dark. These latter results indicate that the increase in growth noted above required evoked action potentials. Together, these results indicate that neural activity regulates the pattern and degree of motor axon growth *in vitro*. Furthermore, they support the notion that drugs altering neuronal activity during fetal development can affect the formation of neuronal circuitries resulting in developmental defects. Currently, steps are being taken in the lab to better understand the intracellular signaling mechanisms responsible for the activity-induced enhancement in neurite growth.

Disclosures: E.K. Aubrey: None. **V.F. Rafuse:** None.

Poster

688. Homeostatic Synaptic Plasticity and Presynaptic Function

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Topic: B.08. Synaptic Plasticity

Support: HD032571

NS057190

Title: Synaptic inputs to axotomized motoneurons are maintained by repeated but not single application of brief electrical stimulation

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Abstract: Following peripheral nerve transection, synapses are withdrawn from the somata of the injured motoneurons. Eventually many of these synapses will be restored, but those expressing vesicular glutamate transporter 1 (VGLUT1) and arising mainly from stretch-sensitive primary afferent neurons are lost permanently. If mice are treadmill trained for two

weeks after injury no withdrawal of synapses is observed. To evaluate whether this effect of exercise is the result of the increased neuronal activity associated with treadmill walking, we transected the common fibular nerve of C57B6 mice bilaterally and then stimulated the right sciatic nerve proximal to the transection for one hour at 20 Hz at an intensity twice that needed to evoke a twitch in the innervated gastrocnemius muscle. Two weeks later the mice were euthanized and tissue sections of the L4-L5 spinal cord segments were reacted with antibodies to VGLUT1 or glutamic acid decarboxylase 67 (GAD67), a marker of inhibitory synapses. The proportion of the somata of axotomized common fibular motoneurons on both sides of the spinal cord which were in contact with VGLUT1+ and GAD67+ synaptic terminals was determined from confocal images of these sections. A single application of brief electrical stimulation (ES) resulted in no significant effect on the withdrawal of synapses. On both the right and left sides of the spinal cord, both VGLUT1+ and GAD67+ synaptic coverage was reduced significantly, relative to intact mice and was not significantly different from that measured in sections from unstimulated but nerve transected controls. If ES was applied for one hour every third day during the two week period, coverage by VGLUT1+ and GAD67+ terminals onto axotomized motoneurons on the right (stimulated) side of the spinal cord was not significantly different from that found in intact controls and was significantly greater than unstimulated controls. On the left (unstimulated) side of the spinal cord, this repeated ES had no effect on synaptic coverage. These findings are consistent with an activity-dependent rescue of synaptic inputs onto injured motoneurons.

Disclosures: A.W. English: None. **C. Liu:** None.

Poster

688. Homeostatic Synaptic Plasticity and Presynaptic Function

Location: Halls A-C

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Program#/Poster#: 688.18/B40

Topic: B.08. Synaptic Plasticity

Title: Visualizing munc13-dependent facilitation of synaptic release by cryo-electron tomography

Authors: *U. DITTMANN, S. ASANO, Y. FUKUDA, W. BAUMEISTER, V. LUCIC; Max-Planck-Institute for Biochem., Martinsried, Germany

Abstract: In presynaptic terminals synaptic vesicles are embedded in a dense filamentous network known as the presynaptic cytomatrix, which is particularly complex in the vicinity of

the active zone, the stretch of cell membrane where vesicle fusion takes place. By means of cryo-electron tomography (cryo-ET) we analyzed vitrified and fully hydrated mammalian presynaptic terminals (synaptosomes) to visualize the main constituents of this cytomatrix: short filaments that link vesicles to each other ("connectors") or to the active zone ("tethers"). Our earlier analysis indicated that the exact configuration of tethers determines vesicle availability for release. Among the proteins of the presynaptic cytomatrix are candidates to play major roles in short-term synaptic plasticity, especially Munc13. We analyzed by cryo-ET presynaptic terminals treated by pharmacological agents known to affect Munc13. Our data shows quantitative changes in synaptic vesicle and tether location and number. This result concurs with the increase of glutamate release from pharmacologically treated synaptosomes and simultaneously indicates that a state of facilitation can be visualized here.

Disclosures: U. Dittmann: None. **S. Asano:** None. **Y. Fukuda:** None. **W. Baumeister:** None. **V. Lucic:** None.

Poster

688. Homeostatic Synaptic Plasticity and Presynaptic Function

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Topic: B.08. Synaptic Plasticity

Support: NINDS R01079307

NINDS 1F32NS086164

Title: Bi-directional homeostatic control of presynaptic neurotransmitter release

Authors: *M. GAVIÑO, G. DAVIS;
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Abstract: Homeostatic signaling systems that stabilize neural function require bi-directional control. The *Drosophila* NMJ has emerged as a model system to study an evolutionarily conserved form of homeostatic control, acting upon presynaptic neurotransmitter release. At this NMJ, inhibition of postsynaptic neurotransmitter receptor sensitivity induces a homeostatic increase in presynaptic neurotransmitter release, a process termed presynaptic homeostatic potentiation (PHP). An opposing process has also been identified whereby an increase in the amplitude of spontaneous miniature release events is offset by a decrease in presynaptic

neurotransmitter release, a process termed presynaptic homeostatic depression (PHD). Although we have gained insight into the molecular mechanisms that achieve PHP, very little is known regarding PHD. We describe here the cellular basis for PHD. We demonstrate that PHP and PHD can co-exist at a single nerve terminal. We then show that whereas PHP requires RIM-dependent modulation of the readily releasable vesicle pool, PHD does not. Furthermore, other mutations that disrupt PHP have no effect on PHD. Finally, we demonstrate that animals expressing PHD have decreased presynaptic calcium influx and reduced CaV2.1 calcium channel abundance at presynaptic release sites, suggesting a model in which PHD is achieved through regulation of CaV2.1 channel levels. We conclude that PHP and PHD rely upon distinct molecular signaling pathways that converge upon the presynaptic CaV2.1 calcium channel to achieve rheostat-like, bi-directional control of neurotransmitter release.

Disclosures: **M. Gaviño:** None. **G. Davis:** None.

Poster

688. Homeostatic Synaptic Plasticity and Presynaptic Function

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01 MH099557

NIH Grant U54 NS083925

Title: The activity-dependent synaptic growth at the *Drosophila* neuromuscular junction depends on the PKA/synapsin pathway

Authors: *C. L. TORRES FERRERIS, A. VASIN, M. BYKHOVSKAIA;
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Abstract: *Drosophila* neuromuscular junction provides an excellent model system to study activity-dependent formation of new synapses, since the growth of new synaptic boutons in this preparation can be robustly and rapidly induced by patterned depolarization. We took advantage of this preparation to investigate how the protein kinase A (PKA) dependent pathway affects the activity-dependent structural plasticity. Confocal imaging of *Drosophila* larvae with GFP-tagged neuronal membranes was employed to monitor the outgrowth of new boutons. To induce outgrowth, the preparations with cut axons were stimulated by three spaced high K^+

depolarizations. To activate the PKA-dependent pathway, the preparations were treated with forskolin. We found that the forskolin pre-treatment produced approximately 50% increase in the synaptic growth. We then tested the hypothesis that this mechanism is mediated by a presynaptic phosphoprotein synapsin, which represents one of the PKA targets. Synapsin mediates synaptic vesicle clustering, plasticity, and neuronal development. We took advantage of synapsin knockout (Syn(-)) *Drosophila* to investigate how synapsin affects synaptic growth and whether its action is PKA-dependent. We found that synaptic growth in Syn(-) preparations is reduced by approximately 30%. Furthermore, in Syn(-) preparations forskolin pretreatment failed to produce any significant enhancement in the outgrowth of synaptic boutons. Our results suggest that the rapid initial growth of new synapses in response to intense activity is mediated via a pathway which depends on the PKA phosphorylation of synapsin.

Disclosures: C.L. Torres Ferreris: None. **A. Vasin:** None. **M. Bykhovskaia:** None.

Poster

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Location: Halls A-C

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Program#/Poster#: 688.21/B43

Topic: B.08. Synaptic Plasticity

Title: Elevation of synapsin IIb levels strongly correlates with submissive behavior

Authors: *E. NESHER[1,2], M. GROSS[1], T. TIKHONOV[1], M. BAIRACHNAYA[1], I. KOMAN[1], G. YADID[2], A. PINHASOV[1];
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Abstract: Synaptic plasticity's influence upon social interactions is widely studied. Previously it was demonstrated that synapsin genes are involved in the regulation of social behavior and that targeted deletion of synapsin genes in mice leads to cognitive impairments and deficits in social interaction. A central pattern of social interactions are relationships in which one member of a group achieves dominant status in relation to its submissive counterparts. Extreme expression of dominant and submissive behavior in animals simulates personality and affective disorders in humans. By employing selective breeding based on a social interaction paradigm, we developed lines of animals with strong features of dominance (Dom) and submissiveness (Sub). We hypothesize that behavior of selectively bred Dom and Sub mice is mediated by changes in synaptic plasticity. We found that the synaptic gene Synapsin (Syn) II was expressed differentially in the hippocampus, striatum and prefrontal cortex of Dom, Sub and Wild Type

mice. These significant changes were attributed to the Syn IIb isoform, but not Syn IIa. Both mRNA and protein levels of Syn IIb are significantly upregulated in the tested brain regions of both Sub males and females. Changes in behavioral status of Sub mice resulting from pharmacological or physiological interventions were accompanied with changes in Syn IIb expression. Thus, behavior of Sub Dams was altered after delivery making them more dominant in comparison to nulliparous Sub females. These behavioral changes were accompanied with decreased Syn IIb expression, which reached the levels of Dom and WT mice. Mating also reduced Sub males' submissive behavior, accompanied by reduction in Syn IIb expression. Moreover, acute administration of paroxetine (3 mg/kg) significantly downregulated the expression of Syn IIb in Sub mice, reaching the levels observed in vehicle-treated Dom animals. Thus, our study clearly demonstrates that changes in Syn IIb expression is correlated with submissive behavioral status and is strongly influenced by antidepressant treatment. Such findings identify Syn IIb as an important regulator of social interaction and further study will evaluate the regulatory mechanisms involved in the altered expression of Syn IIb in the context of submissive behavior.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant Number NINDS R01NS059867

Title: Identification of a presynaptic innate immune receptor required for the homeostatic modulation of neurotransmitter release

Authors: *N. HARRIS[1], D. J. BRASIER[2], D. K. DICKMAN[2], G. W. DAVIS[2];
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Abstract: Given the complexity of the nervous system and its capacity for change, it is remarkable that robust, reproducible neural function and animal behavior can be achieved. The *Drosophila* neuromuscular junction has emerged as a powerful model system for discovering the molecular basis of homeostatic plasticity. In this system, impaired post-synaptic glutamate

receptor function, achieved either pharmacologically or genetically, initiates a homeostatic, compensatory increase in presynaptic neurotransmitter release that restores synaptic depolarization of the muscle cell to baseline levels. This is referred to as presynaptic homeostasis and necessitates a retrograde signal from the muscle to the presynaptic nerve terminal. Recent publications have shown that the increase in presynaptic release observed during synaptic homeostasis requires modulation of both presynaptic calcium influx and the number of readily releasable vesicles (RRP). To date, the signaling system that controls these parameters remains poorly understood, in particular the nature of signaling from muscle to nerve. An electrophysiology-based forward genetic screen has identified several candidate homeostatic plasticity genes. This screen identified mutations in a transmembrane receptor previously characterized within the innate immune system. The innate immune system is conserved in all organisms. The receptor that we identify is a peptidoglycan recognition protein (PGRP). Here, we show that PGRP is required in motor neurons for both the rapid induction and sustained expression of presynaptic homeostasis. Loss of PRGP has no effect on active zone number or the anatomical growth of the motor neuron. In addition, there is no substantive effect on baseline neurotransmission. Finally, we provide evidence that this receptor can be trafficked to the presynaptic nerve terminal where it appears to reside at or near the plasma membrane. Taken together, our data suggest that PGRP is a presynaptic receptor that is required for presynaptic homeostasis, potentially acting as a receptor for a trans-synaptic retrograde signal.

Disclosures: **N. Harris:** None. **D.J. Brasier:** None. **D.K. Dickman:** None. **G.W. Davis:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH NS42599

NIH GM077569

Title: A novel role for NSF in synaptic homeostasis at the *Drosophila* neuromuscular junction

Authors: ***A. P. MULLIN**[1], A. GOKHALE[1], S. SANYAL[2], V. FAUNDEZ[1];
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Abstract: Synaptic activity must constantly be fine tuned in order to maintain appropriate firing properties in response to changes in the synaptic environment. The process by which a presynaptic cell modulates its activity in response to postsynaptic feedback is known as synaptic homeostasis, and allows the cell the retain appropriate synaptic activity within a target range. For example, at the neuromuscular junction in *Drosophila*, animals incubated for 10 minute incubation with philanthotoxin (PhTx), which irreversibly binds non-NMDA glutamate receptors, increase the quantal content of their synaptic release to compensate for the diminished postsynaptic receptor availability and activation. Mutations in genes associated with neurodevelopmental disorders also impair synaptic homeostasis, although the molecular interactions of these genes and their gene products to regulate this phenotype are largely unknown. Recently, loss-of-function mutations in the schizophrenia susceptibility factor dysbindin were shown to preclude philanthotoxin-induced synaptic homeostasis at the *Drosophila* neuromuscular junction. Apart from a well-established role as one of eight subunits comprising the biogenesis of lysosome related organelles complex 1 (BLOC-1), the molecular interactions of dysbindin are largely unknown. To address this question and gain a better understanding of the role for the dysbindin-containing BLOC-1 in synaptic transmission, our lab generated the dysbindin protein-interaction (interactome) network using human neuroblastoma cells. Within this interactome, we identified several components of synaptic vesicle fusion machinery, including the n-ethyl maleimide sensitive factor (NSF). We confirmed the biochemical interaction of dysbindin with NSF, as well as the other fusion machniery components. Additionally, we confirmed that loss of dysbindin in multiple mammalian cell types corresponds with a significant decrease in cellular NSF. Thus, we predicted that NSF and dysbindin exist in an evolutionarily conserved cellular pathway to regulate synaptic vesicle fusion. To test this hypothesis, we assessed the role for NSF in synaptic homeostasis at the *Drosophila* neuromuscular junction. Presynaptic overexpression of NSF in dysbindin loss-of-function flies was able to rescue the dysbindin block on synaptic homeostasis. Our results suggest that interactions between protein complexes within a biochemically defined network regulate complex neural phenotypes, such as synaptic homeostasis.

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Poster

689. Synaptic Plasticity: Transcription and Translation

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Topic: B.08. Synaptic Plasticity

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SFARI #236390

Sloan Foundation

Title: Characterization of locally translated presynaptic proteins

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Abstract: Presynaptic translation is necessary in some contexts of synaptic plasticity. In the invertebrate *Aplysia* long term facilitation in cultured sensory neurons can be elicited after removal of the presynaptic cell body but not following presynaptic application of the translation inhibitor anisomycin. In rodent slice preparations LTD at corticostriatal synapses and LTP at hippocampal mossy fiber-CA3 synapses require presynaptic translation. The evolutionary conservation of presynaptic translation suggests it may play a key role in spatiotemporal adaptation of synapses and we are only beginning to mechanistically understand this process. To identify putative mRNA targets of local translation we performed RNA-seq on axonally localized RNAs from dissociated embryonic mouse hippocampal neurons cultured in 2-compartment microfluidic devices which isolate axons from soma. We detected 109 transcripts encoding known presynaptic proteins, such as B-catenin, complexin and Vamp2, localized to axons with high confidence in 3 of 3 independent biological replicates. These transcripts represent 23% of the known presynaptic proteome. Results were validated by RT-PCR and immunocytochemistry. We investigated endocytic, exocytic and synapse assembly targets following presynapse formation onto PDL-coated beads in the presence and absence of pharmacological inhibitors of translation. Thirty-five of our presynaptic proteome mRNAs are published targets of FMRP, an RNA binding protein and translational repressor, suggesting that FMRP might regulate their translation presynaptically. Characterizing presynaptically produced proteins, the contexts in which they are translated and their local function in synaptic plasticity will augment our mechanistic understanding of presynaptic plasticity.

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Poster

689. Synaptic Plasticity: Transcription and Translation

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Topic: B.08. Synaptic Plasticity

Support: K12HD073945

Sloan foundation

Title: Long-term synaptic plasticity is induced in hippocampal neurons following distal axonal injury

Authors: *T. NAGENDRAN[1,2], R. L. BIGLER[3], R. LARSEN[4,2], B. D. PHILPOT[4,2,5], A. M. TAYLOR[1,2,5];
[1]UNC/NCSU Joint Dept. of Biomed. Engin., [2]UNC Neurosci. Ctr., [3]Curriculum in Genet. and Mol. Biol., [4]Dept. of Cell Biol. and Physiol., [5]Carolina Inst. of Developmental Disabilities, Univ. Of North Carolina, Chapel Hill, NC

Abstract: In the central nervous system (CNS) pyramidal neurons extend single axons long distances to form connections with other neurons; these axons are particularly susceptible to structural damage or stress in neurodegenerative diseases, such as Alzheimer's disease, and in brain and spinal cord injuries and stroke. fMRI studies show that distal spinal cord injury induces cortical reorganization and increases activity in motor cortex, suggesting that distal injury promotes plasticity in long projection pyramidal neurons. Similar enhancements in excitability have been reported in unaffected brain regions following stroke. While injury-induced plasticity is widely accepted, essentially nothing is known about the biological mechanisms of plasticity in uninjured areas of CNS. To examine how distal axon injury modifies synapses over time, we used compartmentalized microfluidic devices as an *in vitro* model system to direct axons over 1mm (or 40 times the width of the soma) into an isolated axonal compartment. This *in vitro* system allowed us to perform axotomy without physically disturbing the remainder of the connecting neuronal networks housed within the somatodendritic compartment. We assessed cell viability at both 24h and 48h post-axotomy and showed that cells remain viable at both time points and regenerate axons. We showed axotomized neurons undergo chromatolysis and a decrease in GABAergic terminals compared to uninjured neuron, as demonstrated *in vivo*. RNA sequencing analysis from the somatodendritic compartment 24h following distal axotomy revealed differential expression of transcripts critical to both postsynaptic and presynaptic

function. To examine injury-induced synaptic changes in the somatodendritic compartment, neurons were labeled using retrograde tracers from the axonal compartment; 24h following axotomy, these neurons showed decreases in total dendrite length and spine number, suggesting fewer synapses. To determine if distal axotomy also affects presynaptic inputs onto distally injured neurons, we used FM dyes and showed that synaptic vesicle release probability is significantly enhanced at 48h, but not 24h, post-injury compared to uninjured controls. We confirmed these results using electrophysiology, showing a significant increase in mEPSC frequency 48h post-axotomy. These results indicate that distal axonal injury induces retrograde synaptic changes, altering their excitability, that proceed from postsynaptic to presynaptic compartments and that persists over days. Together the data suggest that these timed series of events induces long-term synaptic remodeling following injury.

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Poster

689. Synaptic Plasticity: Transcription and Translation

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Title: A role for eRNA in neuronal gene expression

Authors: *K. SCHAUKOWITCH, J.-Y. JOO, X. LIU, T.-K. KIM;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Impairment in learning and memory is a well-established cognitive symptom that is manifested in many psychiatric diseases including autism and schizophrenia. Many studies have shown that long-lasting memory formation is mediated by rapid changes in nuclear gene expression in response to learning-induced sensory experience. Despite these findings, there is a significant gap in our knowledge as to how sensory information is precisely translated into epigenetic networks to elicit specific transcriptional outputs. Recently, a novel class of long noncoding RNAs that are transcribed bidirectionally from the enhancers of activity-dependent genes in neurons (eRNAs) has been identified, and we hypothesize that these eRNAs have a regulatory role in the activity-dependent program of gene expression. We have studied the function of eRNAs that are expressed from the enhancer regions of two immediate early genes *Arc* and *Gadd45b*, which have both been implicated in mediating synaptic plasticity. Using a knockdown approach, we found that eRNAs are necessary for the full induction of their target mRNA and protein levels in response to membrane depolarization. eRNAs specifically regulate the early elongation stage of transcription by allowing for efficient release of paused RNA polymerase II (RNAPII) from the promoters of activity-regulated genes. Knockdown of eRNAs results in the retention of one of the RNAPII pausing factors, Negative Elongation Factor (NELF), at the target gene promoter, as well as lower levels of elongating RNAPII. eRNAs are also directly bound to NELF during stimulated conditions, suggesting that eRNAs interact with NELF to facilitate its release from the promoter, thus resulting in efficient and precisely timed target gene activation. We have observed induction of eRNAs in the intact mouse brain in response to seizure and other sensory stimulation, suggesting that they are functionally involved *in vivo* in regulating the experience-induced gene programs that are thought to underlie learning and memory.

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Poster

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Title: Effects of p11 on BDNF-induced changes in dendritic outgrowth and spine formation in rat hippocampal cells

Authors: M. SEO[1], H. CHO[1], L. NHU[2], J. LEE[1,2,3], Y. KIM[1,2,3], *S. PARK[1,2];
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Abstract: Purpose: Recent study suggests that the antidepressant-like effect of BDNF in well-established behavioral models requires p11 (also called S100A10). BDNF modulates neural plasticity which is linked to the cellular actions of antidepressant drugs. In the current study, we investigated whether p11 regulates effects of BDNF on aspects of neural plasticity *in vitro*. Methods: We used primary hippocampal cultures. The expression of p11, dendritic outgrowth, and spine formation in rat hippocampal cells was investigated under toxic conditions induced by B27 deprivation, which causes hippocampal cell death. The levels of p11 were evaluated using Western blot analyses, and dendritic outgrowth and spine formation was assessed using immunostaining. Results: B27 deprivation significantly decreased p11 expression ($p < 0.01$). BDNF (50, 100, and 200 ng/ml) significantly prevented B27 deprivation-induced decreases in levels of p11 in dose-dependent manner ($p < 0.05$ or $p < 0.01$). BDNF (100 and 200 ng/ml) significantly increased the total outgrowth of hippocampal dendrities ($p < 0.01$) and spine number ($p < 0.01$). Moreover, the effects of BDNF on dendritic outgrowth and spine formation were blocked by small interfering RNA (siRNA) for p11 knockdown (all $p < 0.01$). Conclusions: Taken together, these findings suggest that p11 mediates BDNF-induced improvement in neural plasticity.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH grant NS051187

Title: Rapid regulation of Tet1 expression and protein-protein interactions during *in vitro* classical conditioning

Authors: *Z. ZHENG, J. KEIFER;
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Abstract: Epigenetic mechanisms including DNA methylation, demethylation, and chromatin remodeling events are critically involved in the regulation of gene expression underlying learning and memory. The ten-eleven translocation (Tet1-3) family of proteins converts 5-methylcytosine to 5-hydroxymethylcytosine thought to be an intermediate product in an active oxidative demethylation mechanism in DNA. The physiological function of Tet proteins in learning and memory remain to be determined. To assess whether Tet1 protein expression is conditioning-dependent, we first examined Tet1 during different time points after classical eyeblink conditioning using an *in vitro* brainstem preparation from the turtle, Trachemys scripta elegans. Western blot analysis shows that Tet1 protein is significantly increased after 15 minutes of conditioning but declines to naïve values with further conditioning after 25 or 80 minutes of the stimulation protocol. Moreover, coimmunoprecipitation studies indicate that both total CREB and ERK protein interact with Tet1 but with different binding patterns. During conditioning, the total ERK and Tet1 interaction is significantly increased after 15 minutes but decreases with further stimulation of 25 minutes. On the other hand, the interaction between total CREB and Tet1 is detected in naïve preparations and is significantly reduced during conditioning stimulation. We also have observed a conditioning-dependent interaction of MeCP2 with Tet1 such that binding occurs after conditioning. Immunohistochemical staining reveals that Tet1 is present in both the nucleus and cytoplasm (soma and dendrites) of numerous brainstem neurons including the abducens motor nuclei. Whether there is dynamic conditioning-related translocation of subcellular Tet1 protein is under study. These data indicate that Tet1 undergoes rapid, conditioning-dependent changes in protein expression and interactions with other DNA regulatory proteins involved in conditioning. The potential function of these alterations in gene expression during conditioning will be further characterized using ChIP analysis.

Disclosures: Z. Zheng: None. **J. Keifer:** None.

Poster

689. Synaptic Plasticity: Transcription and Translation

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: CONACYT Grant 123625

CONACYT Grant 163235

Title: Glutamate dependent S6 phosphorylation Involvement of AMPA receptors

Authors: *M. ESCALANTE LOPEZ[1,2], M. A. FLORES MÉNDEZ[1,2], L. C. R. HERNÁNDEZ KELLY[1], A. ORTEGA[1];
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Abstract: Glutamate (Glu) the main excitatory neurotransmitter of the Central Nervous System regulates gene expression at the transcriptional and translational levels through the activation of specific membrane receptors and transporters present in neurons and glia cells. A membrane to nucleus singling cascade triggered by this neurotransmitter has been described in cultured cerebellar Bergmann glia cells. Furthermore, It has also been described that Glu receptors activation is linked to a modulation of [^{35}S]-methionine incorporation into newly synthesized polypeptides. In order to gain insight into the signal transduction cascades that participate in this effect, in the present study we characterized the phosphorylation of several components of the translational machinery. Higher eukaryotic ribosomes consist of two subunits designated as 40S (small) and 60S (large) subunits. The 40S subunit comprises a single molecule of RNA, termed 18S rRNA, and 33 proteins; by contrast, the 60S subunit contains three RNA molecules: 5S, 5.8S and 28S rRNA, and 46-47 proteins. Of all of the ribosomal proteins, S6 has attracted much attention since it undergoes inducible phosphorylation. The phosphorylation sites in S6 have been mapped to five clustered residues, Ser235, Ser236, Ser240, Ser244 and Ser247. Nevertheless, Ser236 phosphorylation is the primary phosphorylation site. The kinases responsible of this modification are p70^{S6K} and p90^{RSK}. S6 phosphorylation increases the affinity of 40s subunit for mRNAs and thus facilitates translational initiation. Glutamate exposure of cultured chick cerebellar Bergmann glia cells results in a time-and dose-dependent increase in S6 phosphorylation. Pharmacological characterization of the Glu effect demonstrated the involvement of Ca^{2+}-permeable α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Concerning the signal transduction cascades that mediate the Glu-dependent rpS6 phosphorylation, both the phospahtidyl inositol 3 kinase (PI3K)/mammalian target of rapamycin (mTOR) and the extracellular regulated kinase (ERK) cascade are critically involved. Finally, phosphorylated rpS6 is mostly detected in the cytoplasmic cell compartment. These results strengthen the notion of an important role of glia cells in Glu-dependent translational control in the cerebellum.

Disclosures: **M. Escalante Lopez:** None. **M.A. Flores Méndez:** None. **L.C.R. Hernández Kelly:** None. **A. Ortega:** None.

Poster

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NSF PRFB DBI-1306528 (F.N.)

Title: Rapid repression of the mechanistic/mammalian target of rapamycin complex 1 (mTORC1) shifts the regional expression of disease-related protein ensembles

Authors: *F. NIERE[1], S. NAMJOSHI[1], E. SONG[2], Y. MECHREF[2], K. RAAB-GRAHAM[1]; [1]Neurosci., Univ. of Texas, Austin, Austin, TX; [2]Chem. and Biochem., Texas Tech. Univ., Lubbock, TX

Abstract: A substantial body of work has determined the molecular players and pathways that give rise to mTORC1-associated diseases. Because activation of mTORC1 is at the core of protein synthesis, mTORC1-associated disorders are generally regarded to be diseases of elevated mRNA translation. However, evidence is emerging that suppression of mTORC1 signaling is equally important in promoting protein synthesis. Of note, suppression of mTORC1 is necessary for the local synthesis of the voltage-gated potassium channel Kv1.1 only in dendrites. We hypothesized that regions or sites of dysregulation (e.g. presynaptic, postsynaptic, mitochondria, endoplasmic reticulum) where protein syntheses take place can contribute to the etiology of mTORC1-associated diseases. Defining these localized, mTORC1-responsive proteins is valuable as they may potentially serve as target- and site-specific therapies for diseases of aberrant mTORC1 activity. Using tandem mass spectrometry, we have found that the global protein networks between the cortices of control and rapidly reduced mTORC1 appear strikingly similar. However, proteins that co-fractionated with postsynaptic density-95 protein (PSD95) generally responded with greatest sensitivity to acute mTORC1 suppression. We have also determined several proteins whose expressions are increased upon a brief repression of mTORC1 signaling in a region-specific fashion (Kv1.1-like proteins) and are implicated in Alzheimer's disease, epilepsy, and autism.

Disclosures: F. Niere: None. **S. Namjoshi:** None. **E. Song:** None. **Y. Mechref:** None. **K. Raab-Graham:** None.

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Topic: B.08. Synaptic Plasticity

Title: The role of BRD4 in neuronal gene expression

Authors: *X. LIU, H.-C. HUANG, T.-K. KIM;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: BRD4 is a member of the bromodomain and extraterminal (BET) subfamily of human bromodomain proteins. The bromodomain is a protein domain that recognizes acetylated lysine residues. This property enables BRD4 to interact with acetylated histones and remain associated with chromatin during mitosis, functioning as a gene bookmarker, which is responsible for accelerating post mitotic gene activation. In addition, BRD4 is shown to promote gene expression through its ability to associate with Mediator complex and P-TEFb complex. Although there are intensive studies on BRD4 function in proliferating cells, little is known about its function in neurons, which are terminally differentiated cells, yet expressing high levels of BRD4. To investigate the role of BRD4 in neuronal gene expression, we performed BRD4 ChIP-seq during the course of gene activation in neurons and found that BRD4 occupies more than 3000 promoters and 600 enhancers after neuronal depolarization. RNA-seq analysis further showed a strong correlation between the levels of BRD4 binding and RNA induction. JQ1 is a cell-permeable small molecule that binds competitively to acetyl-lysine recognition motifs, or bromodomains, and exhibits high potency and specificity towards a subset of human bromodomains including BRD4. Treating neurons with JQ1 dramatically altered activity-dependent expression of IEGs. We are currently conducting BRD4 ChIP-seq in neurons that have been treated with JQ1 to see if the effect of JQ1 in neuronal gene expression is mediated by blocking BRD4 binding to its cognate targets. To further explore BRD4 function in the brain, long-term memory consolidation of mice treated with JQ1 is also being examined.

Disclosures: **X. Liu:** None. **H. Huang:** None. **T. Kim:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: Bioinformatic identification of genes targeted by microRNA regulated following induction of long-term potentiation *in vivo*

Authors: G. JOILIN[1], D. GUEVREMONT[1], W. ABRAHAM[2], C. PRINT[3], *J. M. WILLIAMS[1];
[1]Anat., [2]Psychology, Univ. of Otago, Dunedin, New Zealand; [3]Mol. Med. and Pathology, The Univ. of Auckland, Auckland, New Zealand

Abstract: Long-term potentiation (LTP) is widely studied as the cellular correlate of the mechanisms underlying memory formation. LTP can last for long periods when induced at hippocampal perforant path synapses in awake freely moving animals. Underlying this remarkable persistence are a number of highly regulated complex gene networks. Interestingly, our genomic and bioinformatic analysis predicted that specific networks are likely to be regulated by microRNA, negative regulators of translation. As LTP is characterised by a rapid up-regulation in mRNA expression, we hypothesised that this would occur concurrently with a rapid down-regulation in microRNA expression. Following Affymetrix microRNA profiling and qPCR we confirmed down-regulation of miR-132 and miR-34a (miR-132: 0.37±0.10, $p<0.01$; miR-34a: 0.44±0.07, $p<0.01$; average fold change ± SEM, one sample t-test). To understand the biological significance of down-regulating miR-132 and miR-34a in LTP, it is important to establish their mRNA targets. Current experimental techniques do not allow for efficient and mass identification of the targets of microRNAs, so various computational techniques have been developed. Modelling various characteristics of microRNA:mRNA interactions, such as seed size, thermodynamics, and site accessibility, these algorithms search transcriptome databases to list potential target mRNA meeting set criteria. This approach yields long lists of putative microRNA targets that have little overlap with each other and may not be relevant to the biology under study. Consequently, these lists are manually curated, introducing bias into the discovery of mRNA targets. In order to systematically identify miR-132 and miR-34a target mRNA of likely relevance to LTP, we first produced lists of potential targets from nine microRNA target algorithms, and created sublists by changing the stringency of the criteria. To identify LTP-related gene sets without manual curation, and determine if an enrichment of these gene sets exists within any of these algorithms, these lists were filtered through KEGG pathways and GO processes. No algorithm was found to be more biased to LTP-related gene sets. Targets of miR-132 and miR-34a predicted by four or more algorithms included the ionotropic glutamate

receptor subunit GRIA2, the protein kinases MAPK1 and MAPK2K1, the GTPase KRAS, and transcriptional co-activator EP300. With substantial literature supporting the involvement of these molecules in the persistence of LTP, this analysis bolsters the hypothesis that post-transcriptional regulation of gene expression by microRNA regulates the longevity of LTP.

Disclosures: G. Joilin: None. **D. Guevremont:** None. **W. Abraham:** None. **C. Print:** None. **J.M. Williams:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: CONACYT Grant 123625

CONACYT Grant 163235

Title: Flouride regulates protein synthesis in cultured Bergmann glia cells

Authors: *A. N. ALAMILLO[1,2], M. FLORES-MENDEZ[2,1], D. RAMIREZ[1], L. C. R. HERNANDEZ-KELLY[1], L. M. DEL RAZO[1], A. ORTEGA[1];
[1]Toxicología, [2]Genética y Biología Mol., Cinvestav, México D.F, Mexico

Abstract: Fluoride is the most abundant halogen in the Earth's crust. In endemic fluorosis areas, intake of water with fluoride levels ≥ 1.5 mg/L can result in neurodegenerative diseases. It has been described that this compound has an important impact in brain development and differentiation that often leads to learning and memory deficits. However, the molecular mechanisms associated to fluoride exposure are far for being established. Bergmann glia cells (BGC) are a type of radial glia, which does not undergo the typical astrocytic conversion that occurs after birth. This cell type is found in the molecular layer of the cerebellum surrounding glutamatergic synapses between parallel fibers and Purkinje cells. BGC participate actively in the removal and recycling of glutamate from the synaptic space. Also, these cells provide neurons with lactate in what now is known as the astrocyte/neuronal lactate shuttle. Therefore, a continuous dialogue between neurons and their surrounding glial cells is important for proper brain function. Taking into consideration that Purkinje cells are target of fluoride-induced degeneration, in this contribution, we hypothesized that the effect of this ion might be the result

of impairment of glutamate neurotransmission and in particular of glia/neuronal interactions. To this end, we used the well-established culture system of chick cerebellar BGC. Exposure of the cultured cells to fluoride concentrations as those found in tap water of contaminated regions, results in a time and dose dependent decrease in [35S]-methionine incorporation into newly synthetized polypeptides. Phosphorylation experiments demonstrated a fluoride-induced increase in the phosphorylation pattern of the eukaryotic elongation factor 2, suggesting that the elongation step of protein synthesis is one of the targets of fluoride toxicity. The kinase responsible for eEF2 phosphorylation is a Ca2+/calmodulin dependent kinase known as elongation factor 2 kinase (eEF2K). Accordingly, we were able to demonstrate a fluoride triggered 45Ca2+ influx. Interestingly enough, the glutamate/glutamine shuttle is also affected by fluoride exposure suggesting that fluoride Central Nervous toxicity might have as main target the surrounding glial cells.

Disclosures: A.N. Alamillo: None. **M. Flores-Mendez:** None. **D. Ramirez:** None. **L.C.R. Hernandez-Kelly:** None. **L.M. Del Razo:** None. **A. Ortega:** None.

Poster

689. Synaptic Plasticity: Transcription and Translation

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Topic: F.02. Animal Cognition and Behavior

Support: KAKEN

Title: Motor training changes the properties of layers II/III neurons in the primary motor cortex and glutamatergic synaptic plasticity

Authors: *H. KIDA[1], Y. TSUDA[1], Y. YAMAMOTO[2], Y. OWADA[2], D. MITSUSHIMA[1]; [1]Systems Neurosci., [2]Organ Anat., Yamaguchi university Sch. of Med., Ube, Yamaguchi, Japan

Abstract: To analyze motor learning-dependent changes in layers II/III neurons in primary motor cortex, we trained rats with a rotor rod test (10 sessions per day). Although the motor performance at the 1st session was low, that was significantly improved at the final session of the1st day of training. Then, rats were further trained up to 2 days (total 20 sessions). To evaluate the changes in the neural properties and glutamatergic plasticity using patch clamp method, we made acute brain slices in untrained, 1-day trained, and 2-days trained rats. In current clamp analysis, 1-day trained rats showed lower (-72.7 mV), but 2-days trained rats showed higher

resting membrane potential (-68.7 mV) than untrained rats (-71.0 mV). Although untrained rats required 303 pA to induce an action potential, 1-day trained (292 pA) or 2-days trained rats (217 pA) required lower current injection. In voltage clamp analysis, 1-day trained rats showed significantly higher AMPA/NMDA ratio and miniature EPSC (mEPSC) amplitude than untrained rats, suggesting an increase in postsynaptic AMPA receptors in the early phase of motor learning. Paired-pulse responses of EPSC were not changed in 1-day trained rats. However, the AMPA/NMDA ratio in 2-days trained rats decreased to the levels in untrained rats. That is probably due to an increase in NMDA current, since 2-days trained rats showed significantly higher mEPSC amplitude and frequency than untrained rats. Bath treatment of CNQX (10 μM) consistently blocked the mEPSC events. Moreover, paired-pulse response of EPSC significantly decreased in 2-days trained rats, suggesting the increase in presynaptic glutamate release at the late phase of learning. These results suggest that dynamic changes in the property and glutamatergic plasticity depending on the phase of motor learning in layers II/III neurons in primary motor cortex.

Disclosures: H. Kida: None. **Y. Tsuda:** None. **Y. Yamamoto:** None. **Y. Owada:** None. **D. Mitsushima:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: KAKEN25110705

KAKEN25460090

Title: BDNF expression through CaMKIIδ3 nuclear translocation in dopaminergic neurons

Authors: *N. SHIODA, K. FUKUNAGA;
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Abstract: We previously reported that dopamine D2 receptor (D2R) stimulation activates calcium/calmodulin dependent protein kinase II (CaMKII)δ3, a CaMKII nuclear isoform, thereby increasing brain-derived neurotrophic factor (BDNF) gene expression. However, the precise mechanism underlying the activation of CaMKIIδ3 remains unclear. Here, we found that

CaMKIIδ3 is directly dephosphorylated by protein phosphatase-1 (PP1) at Ser332 site, thereby promoting nuclear translocation. CaMKIIδ3 is expressed in both cytoplasmic and nuclear compartments, while it translocates into nucleus following co-expression with PP1 in Neuro-2a cells. In addition, CaMKIIδ3 co-expressed with PP1 significantly increased the nuclear CaMKII activity and BDNF expression as compared to CaMKIIδ3 alone. Most importantly, administration of dopamine D2R partial agonist, aripiprazole enhanced the CaMKIIδ3 translocation into nucleus and BDNF expression in rat substantia nigra (SN). We also found that aripiprazole treatment enhances the neurite extension and inhibits cell death in cultured dopaminergic neurons. The observations were associated with the nuclear CaMKIIδ3 translocation by PP1 activity. These results suggest that CaMKIIδ3 is dephosphorylated by PP1 at Ser332, resulting in translocation into nucleus. The nuclear translocated CaMKIIδ3 likely facilitates BDNF expression in dopaminergic neurons.

Disclosures: **N. Shioda:** None. **K. Fukunaga:** None.

Poster

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ERANET 1003/12

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Title: Dynamics and consequences of the regulation of protein synthesis at the elongation phase in cortical neurons

Authors: ***J. W. KENNEY**[1], O. SOROKINA[2], M. GENHEDEN[1], L. J. FOSTER[3], J. D. ARMSTRONG[2], C. G. PROUD[1];
[1]Sch. of Biol. Sci., Univ. of Southampton, Southampton, United Kingdom; [2]Sch. of Informatics, Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Numerous aspects of neurophysiology, such as learning and memory, are modulated by calcium-coordinated regulation of cell signalling cascades. For example, the elongation phase of protein synthesis, one of the most energy intensive processes in cells, is regulated by the calcium/calmodulin dependent elongation factor 2 kinase (eEF2K). Furthermore, recent work has found that modifying eEF2K function results in learning and memory deficits in mice. However, little is known about either the dynamics of eEF2k regulation or the consequences of modulating eEF2k function. To analyse the network of biochemical interactions necessary to describe the calcium-coordinated dynamics of eEF2k regulation in neurons, we used a combination of pharmacological, biochemical, and mathematical modelling approaches. In doing so, we identify the MEK/ERK (extracellular regulated kinase), mTORC1 (mammalian target of rapamycin complex 1), and AMPK (AMP kinase) signalling pathways as necessary to explain the regulatory dynamics of eEF2K in neurons, and generated the first mathematical model of eEF2K regulation. Altering mRNA translation at the elongation phase via the modulation of eEF2K activity has been proposed to result in the increased synthesis of specific proteins related to plasticity. However, with the exception of a few well-characterized examples, the identities of these elongation-regulated proteins, and how this regulation is conferred, remains largely unknown. To identify proteins whose synthesis is regulated via eEF2K using an unbiased approach in primary neurons, we developed a novel mass spectrometry based approach in which we combined click-chemistry and SILAC (stable isotope labelling of amino acids in cell culture). Using this approach, we identified several novel candidates whose synthesis is controlled by eEF2K.

Disclosures: J.W. Kenney: None. **O. Sorokina:** None. **M. Genheden:** None. **J.D. Armstrong:** None. **C.G. Proud:** None. **L.J. Foster:** None.

Poster

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Support: NIH grant NS051187

Title: Upstream signaling mechanisms for methylation and regulatory DNA protein binding underlying BDNF gene expression during classical conditioning

Authors: *G. AMBIGAPATHY, Z. ZHENG, J. KEIFER;
Neurosci Group, Basic Biomed. Sci., Univ. South Dakota Sanford Sch. Med., Vermillion, SD

Abstract: Brain-derived neurotrophic factor (BDNF) is a key regulator in synaptic plasticity and learning and is regulated by activity-dependent and tissue-specific promoters. To investigate the epigenetic regulatory mechanisms controlling BDNF expression and function in an *in vitro* model of eyeblink classical conditioning, we analyzed the intracellular signaling molecules that control BDNF gene expression in the pond turtle, Trachemys scripta elegans (tBDNF). The turtle BDNF gene consists of at least three noncoding 5′ exons (I, II and III) with individual promoter regions and one 3′ coding exon (IV). Here we detected DNA methylation levels for exon II and III promoters by bisulfite sequencing PCR (BSP). Our results show that methylation levels were significantly increased in exon II and decreased in exon III after 15 minutes of conditioning (C15) but not after 5 minutes compared to naïve groups. Preincubation with a PDK1 inhibitor, BX-912, inhibits the conditioning related changes in methylation after C15 returning them to naïve levels. This suggests that inhibition of PDK1 affects the process of methylation of exon II and demethylation of exon III promoter regions during conditioning. To understand the mechanisms of epigenetic regulation of the tBDNF gene, we performed ChIP assays for MeCP2 and Tet1 along with the transcriptional activator CREB and repressor BHLHB2. The results show that MeCP2 binding is increased in exon II and decreased in exon III after 15 minutes of conditioning compared with naïve preparations. Tet1 shows opposite effects in exons II and III; binding of Tet1 is decreased in exon II and increased in exon III during conditioning. This is consistent with the high levels of exon II methylation and the demethylation of exon III that occurs during conditioning. Treatment with BX-912 returns MeCP2 and Tet1 protein binding to naïve levels. As expected, the binding of CREB in exon III and BHLHB2 in exon II was significantly increased after 15 minutes of conditioning compared with naïve animals. This conditioning-related increase of CREB and BHLHB2 binding was blocked in BX-912 treated preparations. Additionally, the PKA antagonist Rp-cAMPs inhibits the binding of CREB in exon III and BHLHB2 in exon II during conditioning. Together, these data suggest that MeCP2 and Tet1 modifies the methylation pattern of the tBDNF gene within 15 minutes of conditioning that may facilitate the binding of CREB in exon III to activate transcription and BHLHB2 to repress exon II transcription. These findings also indicate both PKA and PDK1 play a major role in the upstream epigenetic modifications of tBDNF during conditioning.

Disclosures: G. Ambigapathy: None. **Z. Zheng:** None. **J. Keifer:** None.

Poster

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CIHR Operating Grant

Title: TORC1-dependent protein synthesis regulates the excitatory-inhibitory balance and dendritic branching *in vivo* in the retinotectal system of Xenopus laevis

Authors: *D. GOBERT, A. SCHOHL, E. S. RUTHAZER;
Neurol. & Neurosurg., Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: During early brain development, neurons undergo extensive growth and rearrangement of their connections, which ultimately leads to the formation of functional circuits. While TORC1-dependent protein synthesis has been implicated in long-lasting synaptic plasticity in the mature brain, little is known about its role during development. Using electrophysiological recordings and time-lapse two-photon imaging, we investigated the role of TORC1-dependent translation in synapse maturation and stabilization *in vivo* in the retinotectal system of albino Xenopus laevis tadpoles. Here we show that optic tectal neurons in tadpoles raised in rapamycin or electroporated with Raptor antisense MO, to specifically inhibit TORC1, had reduced AMPA mEPSC amplitudes and frequencies, as well as smaller AMPA/NMDA ratios. On the other hand, neurons that were electroporated with Rheb, an upstream activator of TORC1, exhibited greatly enhanced AMPA mEPSC amplitudes and frequencies, as well as larger AMPA/NMDA ratios. Interestingly, GABA mIPSC amplitudes or frequencies were not affected by Rheb expression, resulting in a significant imbalance in the excitatory-inhibitory (E/I) ratio. These results suggest that TORC1 activity is critical to regulate the maturity and the number of excitatory synapses and may contribute to setting the E/I balance. To further investigate how a TORC1-mediated E/I imbalance could affect the cell's integration into the circuit, we performed receptive field mapping of excitatory and inhibitory inputs onto Rheb-expressing tectal neurons. Our preliminary data suggest that TORC1 activation affects the size of spatial receptive fields. Moreover, using *in vivo* single cell electroporation and time-lapse two-photon microscopy, we measured dendritic arbor size and branch numbers in EGFP-expressing tectal neurons and we demonstrated that TORC1 inhibition significantly reduced dendritic arbor size and complexity. In contrast, neurons expressing Rheb had significantly larger and more complex dendritic arbors, suggesting that TORC1 also regulates dendritic branching.

Disclosures: D. Gobert: None. **A. Schohl:** None. **E.S. Ruthazer:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: Changes in molecular phenotype of dorsal root ganglion cells following cruciate ligament transection

Authors: *G. R. MCCORMACK[1], A. G. KANTHASAMY[2], N. D. JEFFERY[3];
[1]Biomed. Sci., [2]Biomed Sci., [3]Vet. Clin. Sci., Iowa State Univ., Ames, IA

Abstract: Changes in Molecular Phenotype of Dorsal Root Ganglion Cells Following Cranial Cruciate Ligament Transection Garrett R McCormack, Anumantha G. Kanthasamy, Nick D. Jeffery The anterior cruciate ligament (ACL) functions as a sensory organ as well as a mechanical stabilizer to the knee. Deficits in proprioception, changes in reflex latencies, and feelings of "giving way" following injury and even reconstruction suggest that this sensory role is vital for the stability and function of the leg and may not always be regained despite adequate mechanical stability. Past research has shown altered knee proprioception in clinical measurements, and delayed latency of the hamstring reflex after ACL injury and treatment. However, molecular and cellular mechanisms underlying neural control changes ofafter ACL injury are not well understood. In this study, we have characterized investigated the long-term phenotypic changes in response to cruciate ligament injury in cells of the dorsal root ganglia (DRG) receiving signals afferent input from the knee joint in response to cruciate ligament injury in an animal model. To this end, the right cranial cruciate ligament (CCL) of adult male Sprague-Dawley rats was transected and, after a three-week recovery period, the lumbar 4 and 5 DRGs from each side were removed. We extracted RNA from the DRG of each side and used a Qiagen RT2 preAMP qPCR array for synaptic plasticity. Gene expression levels on the side ipsilateral to CCL injury were compared to those on the contralateral (control) side. We found upregulation of the genes for NMDA receptor subunit NR2a (Grin2a) and Neurotrophin 4 (Ntf4), and a downregulation of the genes for tumor necrosis factor (Tnf1) and V-rel reticuloendotheliosis viral oncogene homolog A (Rela). These results suggest that alterations in multiple regulatory systems involving glutamate receptors, neuroinflammatory pathway and neurotropic systems may be involved in neural responses to ACL -injury-related regulation of the PNS. Further work, including immunohistochemistry will investigate which cells are expressing these phenotypic changes, how protein content is subsequently changing, and what possible relationships exist between these proteins in the model.

Disclosures: G.R. McCormack: None. **A.G. Kanthasamy:** None. **N.D. Jeffery:** None.

Poster

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Title: Precocious development of visual cortical networks by the removal of molecular restraints leads to dysfunctional vision

Authors: P. LEE[1], R. HERIKSTAD[2], S.-C. YEN[2], *J. SNG[1];
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Abstract: Much of our adult behavior is shaped by experiences during the critical period (CP) in early postnatal development. Since its discovery by Hubel and Wiesel, the visual system has been the classical model for activity dependent neuronal plasticity. We hypothesize that external environment influences the dynamic epigenetic state of the brain and in turn impacts CP plasticity. One epigenetic modification is protein methylation. Protein methylation is catalyzed by enzymes called protein arginine methyltransferases (Prmt). Interestingly, Prmt8 is selectively expressed in the central nervous system (CNS), possibly indicating a special role. Prmt8 is up-regulated in the murine visual cortex, both during developmental CP and experience-dependent when dark-reared. Using a proteome-wide approach (iTRAQ) to compare the synaptic proteins between Prmt8 mutants and their wildtype counterparts, we identified a number of structural proteins to be significantly upregulated in Prmt8 knockout mice. One protein, Tenascin-R (TNR), is a main component of perineuronal nets (PNNs). These nets are implicated in maintaining structural integrity of neuronal networks during the CP. Upon closer inspection of dendritic morphology, Prmt8 knockout mutants display increased spine number and density. This suggests that PRMT8 may act as a molecular brake on structural brakes on plasticity in the visual cortex. Consistent with this hypothesis, removal of PRMT8 causes a drop in strength of visual neuronal responses and visual acuity in these mice, both functionally and behaviorally respectively. Our findings, at this juncture, suggest that PRMT8 plays a role in synaptic plasticity by regulating structural brakes in the brain.

Disclosures: **P. Lee:** None. **J. Sng:** None. **R. Herikstad:** None. **S. Yen:** None.

Poster

689. Synaptic Plasticity: Transcription and Translation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 689.18/C3

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS066583

NIH Grant NS081978

Title: Proteasome modulates transcription-favoring histone modifications at BDNF promoters in long-term synaptic plasticity

Authors: *S. V. BACH, P. R. TACON, J. W. MORGAN, A. N. HEGDE;
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Abstract: The role of the ubiquitin-proteasome pathway (UPP) in long-term synaptic plasticity has been a major focus of many recent studies. The canonical UPP role in degrading regulatory proteins that modulate synaptic plasticity in the cell body or at synaptic terminals is a vast area of current research. The nuclear role of the proteasome in long-term synaptic plasticity has not been studied, however. Several studies in yeast and non-neuronal cells suggest that the proteasome may have non-traditional roles in which it influences transcription. It has been hypothesised that through their ATPase activity proteasomal subunits may target transcriptional co-activators to promoters of actively transcribed genes. This process is mediated by proteasomal subunits interacting with transcriptional machinery responsible for epigenetic remodeling of histone proteins, namely histone acetylation, methylation, and ubiquitination. We used chemically induced long-term potentiation (cLTP) as a model of long-term synaptic plasticity in the murine hippocampus to study how the proteasome influences gene expression and epigenetic modifications of histone proteins. We showed that inhibition of the proteasome prior to cLTP induction prevents upregulation of brain-derived neurotrophic factor (BDNF), a gene essential for synaptic plasticity and memory. Furthermore, we demonstrated that transcription-favoring epigenetic tags (histone H3 acetylated at lysine 9 and 14; histone H3 trimethylated at lysine 4; and histone H2B ubiquitinated at lysine 120) are enhanced after cLTP induction in a proteasome-mediated manner. Finally, we showed that proteasomal ATPase subunits interact with BDNF promoters after cLTP induction. Taken together, our data suggest that proteasomal subunits act as transcription co-factors that guide gene expression by modulating epigenetic events. Our study unveiled a novel role of the proteasome in transcriptional regulation of plasticity-related genes.

Disclosures: **S.V. Bach:** None. **P.R. Tacon:** None. **J.W. Morgan:** None. **A.N. Hegde:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIDA

Title: Impaired responsiveness to cocaine in mice lacking the translin/trax RNAse complex implicated in processing microRNA

Authors: X. FU[1], D. FUKUDOME[1], M. NIWA[1], A. SAWA[1], Y. CHERN[3], *J. M. BARABAN[2]; [1]Johns Hopkins Univ. Sch. Med., Baltimore, MD; [2]Johns Hopkins Univ. Sch. Med., BALTIMORE, MD; [3]Inst. of Biomed. Sci., Academica Sinica, Taipei, Taiwan

Abstract: Translin and trax are homologous proteins that form an RNAse complex enriched in brain neurons. Although recent studies have implicated this complex in processing small RNA species including microRNAs, its physiological role in neuronal signaling is still unclear. As microRNA pathways have been found to regulate dopamine signaling in brain, we have examined whether mice with a deletion of translin (which also leads to loss of trax protein) display altered responsiveness to cocaine. We have found that the ability of cocaine to enhance locomotor activity in an open field arena is reduced by approximately 40% in translin KO mice. Immunostaining of striatal sections revealed prominent nuclear expression of trax in striatal neurons. Examination of trax staining in transgenic mice expressing a fluorescent marker in D1R neurons indicate that trax is expressed in both D1R and D2R striatal neurons. Furthermore, DARPP-32 staining appears to be unaffected in translin KO mice. As these studies demonstrate that the translin/trax complex is expressed in D1R neurons, we are examining whether translin deletion impacts the ability of cocaine to induce transcription of immediate early genes in these neurons. Initial studies indicate that the rapid transcriptional response elicited by cocaine in D1R neurons is impaired, consistent with the locomotor phenotype. Ongoing studies are aimed at assessing whether these impaired responses to cocaine reflect decreased release of dopamine or decreased responsiveness of D1 receptors in translin KO mice.

Disclosures: X. Fu: None. **D. Fukudome:** None. **M. Niwa:** None. **A. Sawa:** None. **Y. Chern:** None. **J.M. Baraban:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: Neuronal-activity and cAMP repress the transcription of Gpr12 to regulate memory formation

Authors: *D. G. WHEELER, K. BAUMGAERTEL, D. ELOW, R. JOHNSON, J. LAPIRA, W. JIANG, R. BARIDO, T. TULLY, R. SCOTT, M. PETERS;
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Abstract: Neuronal activity-dependent transcription in response to behavioral experience plays a critical role in neuronal plasticity and memory formation. Much progress has been made in understanding the signaling pathways that convey signals from the synapse to the nucleus to engage a transcriptional response. However, despite our understanding of how genes are 'turned on' by activity, very little is known about how genes are 'turned off' by activity-dependent transcriptional repression, what genes are down-regulated, and how such down-regulation may impact behavior. To identify genes that are down-regulated by cAMP elevation and synaptic activity, we performed RNA-Seq experiments on primary cultured neurons and discovered that expression of the orphan g-protein coupled receptor, Gpr12, was markedly down-regulated. Gpr12 mRNA stability was unaffected by neuronal activity, but RNA PolII occupancy of the Gpr12 promoter was decreased by cAMP, indicating that activity-dependent decreases in transcription *per se* caused the decrease in Gpr12 mRNA levels. Consistent with these *in vitro* findings, hippocampal Gpr12 mRNA was decreased after contextual fear conditioning *in vivo*. To examine the functional consequence of this activity-dependent down-regulation of Gpr12 mRNA, we examined memory formation in Gpr12 heterozygous knock-out mice, which have a 50% decrease in Gpr12 mRNA levels relative to wild-type littermates. These mice showed enhanced long-term memory in novel object recognition and contextual fear conditioning tasks. Furthermore, RNA interference-mediated knockdown of Gpr12 in the adult hippocampus was sufficient to enhance long-term memory. Together, these results link activity-dependent transcriptional repression of Gpr12 to the formation of long-term memories.

Disclosures: **D.G. Wheeler:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **K. Baumgaertel:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **D. Elow:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **R. Johnson:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **J. Lapira:** A. Employment/Salary (full or part-time):;

Dart NeuroScience. **W. Jiang:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **R. Barido:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **T. Tully:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **R. Scott:** A. Employment/Salary (full or part-time):; Dart NeuroScience. **M. Peters:** A. Employment/Salary (full or part-time):; Dart NeuroScience.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant AG025970

NS21072

Title: Withdrawal of BDNF leads to changes in expression of genes involved in synaptic function

Authors: *A. MARIGA[1], S. GINSBERG[2], I. NINAN[3], M. V. CHAO[4];
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Abstract: Neurotrophins are essential for the development, survival and plasticity of neurons. Reduced neurotrophic factor support has been proposed as one of the early events associated with neurodegenerative and neuropsychiatric disorders. In particular, exogenous delivery of Brain-Derived Neurotrophic Factor (BDNF) can rescue cognitive deficits in animal models of Alzheimer's and Huntington diseases. Despite the overwhelming evidence that BDNF levels are reduced in neurodegeneration, it remains unclear whether low levels of BDNF are a cause, or an effect, of the progressive neuronal loss in vulnerable cell types. To understand the interplay between low BDNF levels and susceptibility to degeneration, we sought to determine the molecular changes that occur as a result of depriving BDNF from primary hippocampal neurons. We were interested in investigating early transcriptional events that occur within 12 hrs following withdrawal of endogenous BDNF with TrkB ligand scavenger (TrkB-FC). Using a high-density microarray platform, we identified significant changes in genes that are associated with vesicular trafficking (Vamp4, Golga5, Rab8b) and synaptic function (Narp, Spry2,

Aggrecan). One of the genes that was significantly reduced in expression is the gene coding for Neuronal Pentraxin 2 (NP2). NP2 is a secreted synaptic protein that is important for excitatory synaptogenesis. Further characterization of NP2 in response to BDNF changes revealed that it is regulated by BDNF. Moreover, preliminary results suggest that NP2 has a functional role in synaptic transmission. Given that one of the early events in neurodegeneration is synaptic loss, these findings are relevant to a wide number of conditions in which levels of BDNF are compromised.

Disclosures: **A. Mariga:** None. **S. Ginsberg:** None. **I. Ninan:** None. **M.V. Chao:** None.

Poster

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Support: Jane Coffin Childs

GM058234

Title: Excitation-transcription coupling via the CaV1-CamKII pathway demands a one-two punch: Ca2+ priming and voltage-dependent capture

Authors: ***M. R. TADROSS**[1,2], B. LI[3], R. W. TSIEN[3,2];
[1]HHMI Janelia Farm, Ashburn, VA; [2]Sch. of Med., Stanford Univ., Palo Alto, CA; [3]Neurosci. Inst., NYU, NY, NY

Abstract: L-type voltage-gated Ca^{2+} channels (Ca_V1) have a privileged role in conveying neuronal electrical activity to nuclear CREB, a transcription factor critical in learning and memory. The mechanism of even the earliest step in this excitation-transcription pathway is not well understood: local Ca^{2+} elevations are thought to be the main trigger in the signaling cascade, but Ca_V1 channels could also impart a voltage-dependent conformational signal (VCS) to nearby signaling intermediates. The role of this VCS has remained enigmatic, owing to a lack of experimental methods to decouple Ca^{2+} influx from the conformational changes required to open the Ca_V pore. To overcome this impasse, we devised an approach where a ligand-gated Ca^{2+}-permeable channel (P2X) is fused to a pore-blocked (non-conducting) Ca_V1, thus enabling Ca_V movement (gated by voltage) to be experimentally uncoupled from local Ca^{2+} influx (gated with

ligand). This molecular dissection uncovered a striking and unexpected requirement for the Ca_V1 VCS in excitation-transcription coupling: we find that Ca_V1 signaling to CREB behaves as an AND gate, where both Ca^{2+} and VCS signals are fundamentally necessary. Mechanistically, we find that this signaling requires both the alpha and beta isoforms of CaMKII, which are recruited to CaV1 channels only when a priming influx of Ca^{2+} is followed by CaV1 voltage-dependent capture. This joint requirement of two temporally distinct signals (i.e., rapid physical VCS movements and slowly varying Ca^{2+} elevations) is reminiscent of the coincidence detection performed by NMDA channels, and may have broad implications for plasticity. Moreover, our methodology may enable independent dissection of Ca^{2+} and VCS signals in a variety of physiological and pathophysiological contexts. To this end, we demonstrate that a widely-studied Ca_V1 Timothy Syndrome point mutation exhibits excessive VCS signaling to both CaMKII and CREB, suggesting that VCS mistuning could play a role in this highly penetrant form of autism.

Disclosures: **M.R. Tadross:** None. **B. Li:** None. **R.W. Tsien:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: Indiana University research funds

Title: Contributions of gonadal hormones to gene expression in adult male rat barrel cortex after peripheral nerve transection

Authors: *J. J. ORCZYK, R. SETHIA, D. LAUGHLIN, P. E. GARRAGHTY;
Psych & Brain Sci., Indiana Univ., Bloomington, IN

Abstract: The goal of this study was to examine changes in the patterns of gene expression in the barrel cortex of adult male rats one day after transection of the infraorbital branch of the trigeminal nerve (ION), and how these changes are affected by orchidectomy. Animals were decapitated 24 hours after ION transection. RNA was then extracted from targeted regions of the cortex and transcriptomes (in excess of 26,000 genes) were generated using RNAseq. Blood for plasma assays of gonadal hormones was collected from all subjects at the time of their sacrifice. In gonadally intact males (N=3), 460 genes showed significant differences in expression levels between the deprived and non-deprived barrel cortices, with roughly half having increased levels

of expression in deprived cortex and roughly half showing decreases. We then selected 227 of these genes for which there were at least an average of 100 total reads across the two hemispheres. Again, roughly comparable numbers of genes showed increases or decreases in expression level in the deprived barrel cortex relative to the intact hemisphere. These genes encode for a wide range of functions, including structural proteins, neurotransmitter receptor subunits, ion channels, and pathology-related processes. In castrated males (N=4), we find differences in the expression of these genes in the two hemispheres to be greatly attenuated or reversed. Importantly, overall reads across the entire transcriptomes of the castrated and gonadally intact males were comparable, suggesting that castration itself did not have a systematic effect on transcription. Rather, castration acted to attenuate or reverse both the increases and the decreases in gene expression found in the sensory deprived barrel cortex of gonadally intact animals. These results demonstrate that sensory loss results in large-scale changes in gene expression in the adult male cerebral cortex. More generally, these results suggest that comparable patterns of changes in gene expression might be expected in any region of cortex that experiences a significant loss of input, as for example, with diaschisis. These results also show that castration, and the resultant disruption in circulating gonadal hormones, profoundly affects these changes in gene expression, a finding that suggests that hormonal status is a significant variable in the design of therapeutic regimens in cases where afferent drive is substantially reduced.

Disclosures: **J.J. Orczyk:** None. **R. Sethia:** None. **D. Laughlin:** None. **P.E. Garraghty:** None.

Poster

689. Synaptic Plasticity: Transcription and Translation

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Topic: F.02. Animal Cognition and Behavior

Support: PO1 NS062686

Title: TrkB signaling in Parvalbumin expressing neurons is required for basket cell connectivity, network function and results in global behavioral deficits

Authors: ***D. XENOS**[1], J. A. CARDIN[2,3], M. L. SCHWARTZ[2], F. M. VACCARINO[1,2,3]; [1]Child Study Ctr., [2]Dept. of Neurobio., [3]Kavli Inst. for Neurosci., Yale Univ., New Haven, CT

Abstract: GABAergic inhibitory interneurons are locally projecting cells that control and synchronize the output of cortical pyramidal neurons. Among them, parvalbumin (PV)-expressing cortical GABAergic neurons, including basket cells which target the pyramidal cell soma and the less abundant chandelier cells which target the axon initial segment, are known to be specifically affected in neurodevelopmental disorders. Strong evidence shows that GABAergic inhibition is under the influence of the neurotrophin BDNF in a region-specific manner, by promoting the expression of GABA-related proteins, regulating GABAergic synapse formation and activity-dependent pruning of their synaptic connections. Neurotrophin-induced effects might be more pronounced for PV-expressing cortical GABA neurons, as TrkB is predominantly expressed by PV-expressing cells. Here, we conditionally ablated the TrkB (Tropomyosin-related kinase B) gene, the main receptor for BDNF, only in parvalbumin positive neurons (PV-cre;TrkB cKO mice), in order to examine potential abnormalities in the postnatal development of these neurons and the circuitry they are involved, as well as behavioral consequences of these effects. We show that PV-cre;TrkB cKO mice displayed profound spontaneous hyperactivity, severe motor coordination impairment and working memory defects. Expression of parvalbumin was reduced in the cortex and in the cerebellar molecular layer of the knockout mice. In the cortex, phosphorylation of MAP kinase 1/2 and the mTOR complex proteins, the major signaling pathways downstream of theTrkB receptor, were decreased. *In vivo* electrophysiological recordings detected reduced oscillatory activity in the gamma range of the local field potential (LFP) in sensory cortex of the knockout mice, associated with altered firing of putative excitatory neurons. No changes were detected in striatal LFP or spiking activity. Interestingly, inhibitory presynaptic terminal boutons onto the cell body of the cortical layer V pyramidal neurons were found to be strongly reduced. Morphological analysis of the cellular architecture of cortical PV cells is currently being performed. The data suggest that (1) normal development of cortical and cerebellar PV interneurons is strongly dependent on TrkB signaling; (2) TrkB is required for cell survival and for normal axon targeting of basked cells during postnatal development; and (3) deficient basket cell targeting of pyramidal cell somata leads to decreased gamma-band oscillations of pyramidal cells and abnormal cognitive behavior.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: R01 NS45324

P30 NS062691

R01 MH609197

Title: Deep sequencing reveals widespread changes in the population of ribosome-associated mRNAs in excitatory neurons following induction of late-phase LTP

Authors: *P. B. CHEN[1], T. J. O'DELL[2], C. BLUM[3], R. KAWAGUCHI[3], G. COPPOLA[4], K. C. MARTIN[1];
[1]Biol. Chem., [2]Physiol., [3]Informatics Ctr. for Neurogenetics and Neurogenomics, [4]Dept. of Psychiatry and Behavioral Sci., UCLA, Los Angeles, CA

Abstract: Synaptic plasticity is the process by which experience changes the strength and number of synaptic connections in the brain. Long-term potentiation (LTP) of hippocampal synapses is a form of synaptic plasticity in which specific patterns of activity trigger increases in synaptic strength; LTP is thought to underlie behavioral learning and memory. New gene transcription and translation during an early critical window are required for the late phase of LTP (L-LTP).[1] To better understand the regulation of gene expression during LTP induction, we performed RNA sequencing (RNA-seq) of two distinct RNA populations post-LTP induction—the total population of transcripts and the transcripts that are loaded onto ribosomes. Towards this end, we employed a transgenic mouse model (the RiboTag mouse)[2] that expresses a hemagglutinin (HA)-tagged ribosomal protein L22 in the presence of Cre recombinase. By crossing the RiboTag mice with a transgenic mouse expressing Cre under the CaMKIIα promoter, HA-tagged L22 was expressed only in excitatory neurons, allowing for purification of the ribosome-loaded population specifically from CA1 and CA3 neurons in hippocampal mini-slices. We observed a significant depletion of non-excitatory neuron specific transcripts in the ribosome-loaded RNA population and detected robust gene expression changes in *c-fos* and *arc*—two immediate early genes important for LTP—following LTP induction in both total RNA and ribosome-associated mRNA. Subsequently, using a transcription and translation-dependent chemical L-LTP induction protocol[3] in acute hippocampal mini-slices, we purified total and ribosome-associated RNA at 30’ and 60’ post-LTP induction, alongside time-matched vehicle-treated controls for RNA-seq. Differential gene expression analysis revealed two striking observations: 1) there is a major burst in transcriptional and translational activity between 30’ and 60’ post-LTP induction and 2) the regulation of translation, as measured by loading of transcripts onto ribosomes, is significantly more dynamic than the regulation of transcription. Specifically, at the 60’ post-LTP time point our RNA seq analysis detects approximately 5,000 genes being significantly differentially expressed in the ribosome-loaded population, as opposed to the approximately 200 observed in the total RNA population. These results shed new light on the temporal dynamics and regulation of transcription and translation during new gene

expression essential for LTP. 1. Barondes and Cohen 1967, *PNAS 2.* Sanz et al. 2009 *PNAS* 3. Chotiner et al. 2003 *Neuroscience*

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Poster

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Title: Endocannabinoid-mediated long-term depression of inhibition requires presynaptic protein synthesis

Authors: *T. J. YOUNTS, M. E. KLEIN, P. E. CASTILLO;
Dept. of Neurosci., Albert Einstein Col. of Med., BRONX, NY

Abstract: Many forms of postsynaptic long-term plasticity rely on protein synthesis; however, it remains unclear and controversial whether translation is needed for presynaptically-expressed, long-term plasticity in the mature mammalian brain. In the hippocampus, endocannabinoid (eCB)-mediated long-term depression of inhibition (iLTD) is known to be expressed presynaptically at a subset of inhibitory interneurons, and previous indirect studies hint that eCB-mediated LTD may require translation. Using selective pharmacology and electrophysiological recordings in acute hippocampal rat slices to monitor inhibitory synaptic transmission, we tested the hypothesis that eCB-mediated iLTD requires presynaptic protein synthesis. When type-1 cannabinoid receptors (CB1Rs) were directly activated with a CB1R agonist, long-term suppression of inhibition was engaged. In contrast, when protein synthesis was acutely blocked with anisomycin or cycloheximide, agonist-induced long-term suppression of inhibition was not observed. This result suggests CB1R activation directly engages the translational machinery. We also examined synaptically-induced eCB-iLTD. Compared with control conditions, both translation inhibitors prevented synaptically-induced eCB-iLTD. Of note, translation inhibitors alone had no lasting impact on basal inhibitory synaptic transmission, suggesting the deficit observed for iLTD was not due to general disruptions in translation. Moreover, neither compound had an effect on short-term eCB-mediated inhibitory plasticity, suggesting eCB

release and detection were not affected by protein synthesis inhibitors. To directly interfere with protein translation in presynaptic or postsynaptic compartments, we performed cell-paired recordings between a presynaptic interneuron and postsynaptic CA1 pyramidal cell and infused a cell membrane-impermeable inhibitor through the recording pipette(s). Remarkably, iLTD was abolished when M7GpppG-an RNA cap analog that blocks translation by competing with initiation factors-was infused into the presynaptic interneuron but not the postsynaptic pyramidal cell. Collectively, we provide direct functional evidence that eCB-LTD requires translation in presynaptic compartments. Our results indicate that the translational machinery previously described in dendrites may also be present in presynaptic terminals, and that certain hippocampal inhibitory interneurons participate in protein synthesis-dependent long-term synaptic plasticity.

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Poster

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Title: Visualization of the newly-synthesized proteins required for synaptic plasticity in Xenopus laevis

Authors: *H.-H. LIU[1,2], T. J. WISHARD[1,3], H. T. CLINE[1,2,3];
[1]Mol. and Cell. Neurosci., [2]Kellogg Sch. of Sci. and Technol., The Scripps Res. Inst., La Jolla, CA; [3]Biol. Sci., The Univ. California, San Diego, La Jolla, CA

Abstract: Synaptic plasticity, the cellular basis of learning and memory, is dynamic at both transcriptional and translational levels. We are interested in how protein synthesis is regulated

with changes in synaptic transmission in response to visual stimuli or disease conditions. Unbiased investigation of global protein synthesis is challenging due to the lack of available techniques. We adapted a new technique, fluorescent non-canonical amino acid tagging (FUNCAT), a nonbiased labeling for newly-synthesized proteins, to examine the distribution of newly-synthesized proteins in Xenopus laevis. The localization of newly-synthesized proteins was ubiquitous in the tadpole brains but the fluorescence intensity varied between different cell populations and the neuropil. The neural progenitors, labeled by SOX2, have higher intensity labeling, indicating that the amount of translation may vary between cell-types in the developing brain. In addition, changes in protein synthesis were detected when animals were exposed to anisomycin, a protein translation inhibitor, and pentylenetetrazol (PTZ), a GABA receptor antagonist, known to elevate brain activity and to induce seizure. Currently, we are using FUNCAT to examine the changes of global protein synthesis in animals, which (1) are exposed to visual stimuli that are known to induce synaptic plasticity or (2) are electroporated with morpholinos to knock down proteins known to regulate protein translation and synaptic plasticity.

Disclosures: H. Liu: None. **T.J. Wishard:** None. **H.T. Cline:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: Department of Biotechnology, India

Core grant To National Brain Research Centre from DBT India

Title: Effects of histone deacetylase inhibitor on massed pattern-induced synaptic plasticity and memory

Authors: *S. K. SHARMA, K. P. SHARMA, K. PANDEY;
Natl. Brain Res. Ctr., Manesar, India

Abstract: At the behavioural level, it is well established that massed training is less effective in inducing long-term memory than the spaced training. At the cellular level, the two patterns of stimulations differentially affect the development of long-term potentiation, which is a candidate

cellular mechanism of memory formation. Several studies have shown that increasing the level of acetylation by inhibition of histone deacetylases facilitates long-term potentiation. Similarly, increasing protein acetylation level enhances memory. However, the effects of increasing acetylation on massed pattern-induced synaptic plasticity and memory is not known. Using rat hippocampal slices, we show that inhibition of histone deacetylases facilitates long-term potentiation induced by massed pattern of stimulation. The inhibition of histone deacetylases has no effect on baseline synaptic transmission or input-output property. Furthermore, at the behavioural level, we show that increasing the level of acetylation facilitates spatial memory induced by massed training. Collectively, the results show that enhancing the level of acetylation has a positive effect on synaptic plasticity and memory formation induced by massed pattern.

Disclosures: S.K. Sharma: None. **K.P. Sharma:** None. **K. Pandey:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NSF Grant 1103738

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Title: Group I mGluR activation leads to an increase in alpha2/delta-2 calcium channel subunit levels

Authors: *L. P. CACHEAUX, F. NIERE, K. RAAB-GRAHAM;
Univ. of Texas at Austin, Austin, TX

Abstract: A fundamental property of neurons thought to underlie learning and memory is the ability to alter synaptic strength through changes in neuronal activity. Protein synthesis is necessary for different types of synaptic plasticity such as long term potentiation (LTP) and long term depression (LTD). LTD mediated by group I metabotropic glutamate receptors (mGluRs) results in rapid protein synthesis in the hippocampus. In this study we identify the calcium channel subunit Cacna2d2 as a new target of mGluR-induced translation. We first verified the presence of Cacna2d2 mRNA at hippocampal dendrites using fluorescent *in situ* hybridization. When group I mGluRs were activated in slices and in hippocampal cultures we observed an

increase in Cacna2d2 protein expression without altering mRNA levels. Furthermore, Cacna2d2 protein levels increase in the dendrites and colocalize with PSD95 to a greater degree after mGluR activation. Since the Fragile X mental retardation protein (FMRP) has been shown to regulate protein synthesis mediated by group I mGluR activation we measured Cacna2d2 protein levels in Fmr1 knockout mice and found can increase suggesting that Cacna2d2 may also be regulated by FMRP. Characterizing the translational regulation mRNAs such as Cacna2d2 will advance the current understanding of how local protein synthesis underlies changes in neuronal excitability and ultimately learning and memory processes.

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Poster

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Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 689.30/C15

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

Title: Characterizing c-fos enhancer function in activity-dependent neuronal gene expression

Authors: *J.-Y. JOO[1], T.-K. KIM[2];
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Abstract: The immediate early gene (IEG) c-fos is widely expressed in the brain and participates in learning and memory. Recently Tae-Kyung Kim and colleagues revealed that five putative c-fos enhancers were identified on the basis of two characteristics of enhancers. One is all five enhancers bind the transcriptional co-activator p300/CBP, the other one is all c-fos enhancers bind histone H3 monomethylated at lysine 4(H3K4me1). Thus a question arises whether five different enhancers regulate c-fos gene expression in the neuron. In this study, we show that each enhancer regulated activity-dependent c-fos gene expression using luciferase assay. The c-fos gene expression is significantly increased at enhancer 1, 2 or 5 transfected neurons with KCl stimulation while only enhancer 1 has strong induction potential in non-neuronal cells. Interestingly each enhancer potential was different by different stimuli. These results suggest that each of the five c-fos enhancers have a specific function in activity-dependent neuronal gene expression.

Disclosures: J. Joo: None. **T. Kim:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.01/C16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 N5046451

Title: Loss of NMDAR-dependent LTP coincides with deficits in spatial working memory in 3xTg-AD mice

Authors: *J. CLARK[1], M. FURGERSON[2], J. D. CRYSTAL[4], M. FECHHEIMER[3], R. FURUKAWA[3], J. J. WAGNER[1];
[1]Physiol. and Pharmacol., [2]Biochem. and Mol. Biol., [3]Cell. Biol., Univ. of Georgia, Athens, GA; [4]Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Alzheimer's disease is a neurodegenerative condition believed to be initiated by production of amyloid beta peptide which leads to synaptic dysfunction and progressive memory loss. In a 3xTg mouse model of Alzheimer's disease (3xTg-AD), we use an 8-arm radial maze with a delayed spatial win-shift procedure and field potential recordings from the CA1 region of hippocampal slices to determine the contribution of NMDA receptor dependent mechanisms of synaptic plasticity in spatial working memory. Our study shows 3xTg-AD mice have a reduction in NMDA receptor dependent LTP and an increase in non-NMDA receptor dependent LTP, leading to a total LTP that is similar between 3xTg-AD and control mice at 3 months of age. Both young (3 mo) and older (8 mo) 3xTg-AD mice exhibited reductions in both paired-pulse facilitation and NMDA receptor dependent LTP that coincide with an impairment in spatial working memory. This impairment correlates with an increase in amyloid beta 42 in 3xTg-AD mice, demonstrating the onset of behavioral and neurophysiological alterations before the detectable presence of plaques and tangles.

Disclosures: J. Clark: None. **M. Furgerson:** None. **J.D. Crystal:** None. **M. Fechheimer:** None. **R. Furukawa:** None. **J.J. Wagner:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.02/C17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R21 NS082870

NIH Grant UL1 RR025758

Title: Cortical plasticity in type-2 diabetes mellitus and its relationship to cognitive decline

Authors: P. J. FRIED[1], L. SCHILBERG[1,3], A.-K. BREM[1,4], N. BOLO[2], H. THÉORET[1,5], *A. PASCUAL-LEONE[6];
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Abstract: Objective. Type 2 Diabetes Mellitus (DM2) is a major cause of disability and death, affecting nearly 26 million people in the US. Nearly 75% with DM2 will develop diabetes-related damage to their nervous system, including behavioral and cognitive deficits and increased risk of dementia. The goal of the present study is to advance our understanding of the neurobiological substrates for these cortical brain consequences of DM2 and develop a reliable assay for their early detection and longitudinal assessment. Consistent with animal models of the effects of insulin and diabetes on brain function, we hypothesized that cognitive dysfunction in DM2 is associated with alterations in cortical brain plasticity. We further hypothesize that these abnormalities can be demonstrated by transcranial magnetic stimulation (TMS) measures. **Methods.** 14 individuals with DM2 (6 males, mean age 65.2 y) and 11 healthy controls (5 males, mean age 66.5 y) have been studied to date. Motor cortical plasticity is evaluated using MR-guided TMS-EMG measures obtained from the right first dorsal interosseus (FDI) muscle and following the Theta Burst Stimulation (TBS) paradigm. Single TMS pulses are applied to assess baseline cortical reactivity. Plasticity is induced with a short regimen of intermittent TBS, after which cortical reactivity is reassessed at regular intervals for 90 minutes. Cognitive functions are assessed with the Alzheimer's Disease Assessment Scale (ADAS-Cog) and the Face Name Associative Memory Exam (FNAME). Subjects' brain-derived neurotrophic factor (BDNF) polymorphism and apolipoprotein E (ApoE) status are assessed and used to parse out the study population given evidence of their impact on plasticity. **Results.** So far, baseline reactivity does not differ between the DM2 and control groups (p = .618). The change in MEP amplitude 10 minutes after iTBS is significantly lower in Val-Val BDNF and ApoE4-negative DM2 subjects

than matched controls (p = 0.005), indicating reduced plasticity. Further, motor cortical plasticity measures were significantly correlated with ADAS-cog (p = .001) and FNAME (p = .047) scores, demonstrating that lower plasticity is associated with lower cognitive functioning. **Conclusions.** Our findings reveal deficits in motor cortical plasticity in DM2 patients as compared to healthy controls. These abnormalities are associated with cognitive dysfunctions. These findings support the utility of TMS-EMG plasticity measures as a surrogate marker for the neurobiological consequences of DM2. Understanding and detecting early neurobiological alterations is crucial in order to prevent and postpone cognitive decline in patients with DM2.

Disclosures: P.J. Fried: None. **A. Pascual-Leone:** None. **L. Schilberg:** None. **A. Brem:** None. **N. Bolo:** None. **H. Théoret:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.03/C18

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: SUVN-G3031: A novel and potent histamine H3 receptor antagonist for potential treatment of cognitive deficits

Authors: R. MEDAPATI, N. MUDDANA, *A. K. SHINDE, P. JAYARAJAN, V. MEKALA, S. IRAPANNANAVAR, V. KANAMARLAPUDI, R. PONNAMANENI, M. FAHEEM, V. GOYAL, S. PANDEY, P. GANGADASARI, R. NIROGI;
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Abstract: Histamine H3 receptors play a critical role as neuromodulators. Blockade of this receptor augments the pre-synaptic release of both histamine and other neurotransmitters including acetylcholine. H3 receptor antagonist enhances the release of histamine which is important for attention and wakefulness. Currently, several H3 receptor antagonists/inverse agonists are in clinical development. Side effects with the current H3 receptor antagonists/inverse agonists are higher, hence novel and chemically diverse H3 receptor antagonists may be devoid of side effects. Here, we describe a novel H3 receptor antagonist. *In vitro* affinity and selectivity profile of SUVN-G3031 was assessed. Pharmacokinetic and brain penetration was evaluated in male Wistar rats. In-vivo receptor occupancy was carried at various dose levels using non-radiolabled tracer in rats. SUVN-G3031 was evaluated in H3 receptor agonist induced dipsogenia and rat models of cognition. Effect of SUVN-G3031 on histamine

and acetylcholine modulation in brain was studied using microdialysis. Toxicity profile of SUVN-G3031 was evaluated in rodents / non rodents and *in vitro* models. SUVN-G3031 is one of the lead molecules with hKi of 8.7 nM and has more than 100 fold selectivity against the related GPCRs. SUVN-G3031 exhibited desired pharmacokinetic properties and brain penetration. This molecule exhibited an excellent separation between H3 affinity and hERG ion channel inhibition. SUVN-G3031 blocked R-α-methylhistamine induced water intake and increased tele-methylhistamine levels in brain and cerebrospinal fluid. Treatment with SUVN-G3031 significantly reversed time induced memory deficit in novel object recognition test and scopolamine induced memory deficit in T-maze & Morris water maze task. A single dose oral administration resulted in H3 receptor occupancy up to 85% in rats and significantly raised acetylcholine and histamine levels in the cortex. SUVN-G3031 was well tolerated in toxicity studies in animals with wide margin of safety and is non mutagenic in *in vitro* assays. SUVN-G3031 displayed desired efficacy, safety, pharmacokinetic and metabolic profiles for further development. IND enabling studies have been completed and US IND filing is in progress.

Disclosures: **R. Medapati:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **N. Muddana:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **A.K. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Mekala:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **S. Irapannanavar:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Kanamarlapudi:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **R. Ponnamaneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **M. Faheem:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **S. Pandey:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **P. Gangadasari:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.04/C19

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The Association of aneuploidy with the development of dementia in neurodegenerative diseases

Authors: *J. CANEUS;
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Abstract: Over the last decades, research has shown that individuals with Down syndrome (trisomy 21) will develop Alzheimer's disease (AD) neuropathology by the age of 40 and that the majority of these individuals will eventually develop dementia. Dementia encompasses many neurodegenerative diseases including AD; however, the mechanism(s) responsible for the development of dementia remain unclear. Down syndrome (DS) is known to be caused pathologically by aneuploidy, in particular three copies of Chromosome 21 called "trisomy 21". Furthermore, studies from our laboratory and others have shown elevated levels of aneuploidy, in particular trisomy 21, both in familial and sporadic Alzheimer's disease. The question then became whether aneuploidy could be a pathological mechanism responsible for the development of dementia in individuals with AD and other neurodegenerative diseases, is significant. In fact, evidence from the Arendt lab has indicated that the death of aneuploidy cells in AD accounts for 90% of neuronal cell loss from the mild cognitive impairment stage to late stage AD. In this study, we analyzed and compared the level of aneuploid cells from brain tissues of three groups, characterized as individuals who were **(1)** diagnosed with AD, **(2)** normal cognition with moderate AD pathology (ADPNC) and **(3)** normal cognition with no AD pathology (control). The data revealed a significant increase of aneuploid cells (trisomy and monosomy for chromosome 12 and 21 in the individuals with AD compared to the Non-demented (ADPNC) and controls, whereas there was no significant difference between the ADPNC and controls. In addition, recent studies have identified mutations in the progranulin (PRGN) gene as a pathological cause for the development of Frontotemporal dementia (FTD), and recently we have shown that FTD patients with the mutant PRGN gene also exhibit elevated aneuploidy. In sum, because these data seem to indicate that aneuploidy is involved in the progression of more than one form of dementia, it is important to understand the full effect of aneuploidy in the development of neurodegenerative disease, the mechanism by which it arises and how to prevent it.

Disclosures: J. Caneus: None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.05/C20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Korea Healthcare Technology R&D Project A111230

National Research Foundation of Korea 2011-0021866

Seoul National University Bundang Hospital Research Fund 03-2010-007

Title: Phloroglucinol rescues the impairment in synaptic plasticity and memory in an animal model of Alzheimer's disease

Authors: *E. YANG, J. RYU, M.-S. CHOI, H.-S. KIM;
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Abstract: Alzheimer's disease (AD) is the most commonly form of dementia in the elderly. Significant pathological hallmarks of AD are amyloid plaques and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein. Both amyloid plaques and tau protein cause the decline of cognition. Impaired synaptic plasticity in neuronal networks is thought to be the underlying cognitive deficits in AD. Oxidative stress (OS) results from an imbalance between antioxidant defenses and the intracellular accumulation of reactive oxygen species (ROS) which contributes to memory deficits. In recent studies, human AD brain have shown in increased in the marker for oxidative stress and high levels of ROS production resulting in negative effects on synaptic plasticity.. However, the mechanisms involved are yet to be elucidated. Therefore, regulating oxidative damages may provide therapeutic efficacy in terms of synaptic plasticity in AD. Phloroglucinol(1, 3, 5 - trihydroxybenzene) is one of the polyphenol group and monomer of phlorotannin. Previously it has been reported that phlorogluicnol reduced cell damage caused by hydrogen peroxide in lung fibroblast cells, as well as gamma ray-induced oxidative stress via antioxidant system. In this study, we found that HT-22 cell line treated with oligomeric amyloid beta(Aβ1-42) has increased ROS production and treatment with phloroglucinol restored ROS levels. In addition we observed phloroglucinol rescues dendritic spine density reduction induced by oligomeric amyloid beta(Aβ1-42). We also found that stereotaxic injection of phloroglucinol in 5-month-old Tg6799 mice attenuates the impairments in cognitive dysfunction observed in 5X FAD(Tg6799) mice, AD animal model. Taken together, these results suggest that phloroglucinol has therapeutic effects by restoring cellular ros levels, and synaptic plasticity and improving cognitive impariments found in 5X FAD mice.

Disclosures: E. Yang: A. Employment/Salary (full or part-time):; Brain korea 21 plus. 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.; Korea Healthcare Technology R&D Project A111230, National Research Foundation of Korea 2011-0021866, Seoul National

University bundang hospital research Fund 03-2010-007. **J. Ryu:** None. **M. Choi:** None. **H. Kim:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.06/C21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research; Grant number: MOP93644

Title: Nature and extent of person recognition impairments associated with capgras syndrome in lewy body dementia

Authors: *C. FIACCONI[1], V. BARKLEY[2], E. C. FINGER[1], N. CARSON[2], D. DUKE[1], S. ROSENBAUM[3], A. GILBOA[4], S. KÖHLER[1];
[1]Univ. of Western Ontario, London, ON, Canada; [2]York Univ., Toronto, ON, Canada; [3]York Univ., London, ON, Canada; [4]Univ. of Toronto, Toronto, ON, Canada

Abstract: Capgras Syndrome (CS) refers to a condition in which patients adopt the delusional belief that a highly familiar individual (e.g., spouse) has been replaced by an imposter. Although traditionally associated with psychiatric illness, recent evidence suggests that CS is often present in neurodegenerative dementia, including Alzheimer's Disease (AD) and Lewy Body Dementia (DLB). Prior psychophysiological research on CS associated with other etiologies has demonstrated reduced autonomic arousal responses to those individuals targeted by the delusion. These findings have led to the suggestion that patients with CS have deficits in covert, but not overt aspects of person recognition. Furthermore, it has been suggested that the deficits observed in CS represent the "mirror image" of those associated with prosopagnosia, in which overt, but not covert person recognition is thought to be impaired. Here, we examine the proposed dissociation between preserved overt and impaired covert recognition responses for CS in the context of DLB more closely. Specifically, we aimed to determine whether overt recognition of famous people would indeed be preserved, despite the presence of CS. Moreover, we examined whether any potential overt person-recognition impairments would be restricted to face cues, or would generalize to other cues, specifically voices and names. A final question regarding the scope of person recognition impairments we addressed was whether such impairments extend beyond person identity and include deficits in overt recognition of facial affect, i.e., facial emotional expressions. We pursued these goals by studying a patient with DLB who showed

clear signs of CS (DLB+) and compared him to another patient with DLB who did not experience CS (DLB-), as well as to a group of healthy control participants. We employed a series of 2-alternative forced choice fame-recognition tasks, and found that performance in the DLB+ patient was impaired relative to DLB- and controls for famous faces and voices, but not for names. Moreover, the DLB+ patient had reduced confidence in those decisions, and generated fewer semantic facts in response to famous faces and voices but not names. To probe affect recognition, we employed a task that required fine-grained judgments of the intensity of fear expressions. Unlike healthy controls and the DLB- patient, the individual with DLB+ provided fear ratings that did not vary with the depicted intensity of fearful expression. Together, these results cast doubt on the strict separation of covert and overt recognition processes, and suggest that CS, at least in the context of DLB, is not a deficit restricted to covert person recognition.

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Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.07/C22

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Impact of chronic NR2B reduction in a mouse model

Authors: *G. RAMMES[1], M. WULF[2], K. KELLERMANN[1], J. DEUSSING[2], C. WOTJAK[2], C. PARSONS[3];
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Abstract: Amyloid β (Aβ) is widely accepted to be one of the major causes of AD accompanied by memory loss and neuronal death. Aβ1-42 is thought to be the most pathogenic form and numerous studies have reported that soluble Aβ1-42 oligomers inhibited long-term potentiation (LTP). It has been shown previously that the pharmacological and transgenic attenuation of NR2B activity reverses the Aβ1-42-induced synaptotoxic effects on LTP. To elucidate whether a permanent reduction of the NR2B subunit affects the pathology of AD, we cross-bred mice heterozygous for NR2B receptor in the forebrain (hetNR2B) with a mouse model for AD carrying a mutated amyloid precursor protein with the Swedish and the arctic mutation (mAPP)

resulting in a hetNR2B/mAPP transgenic. By means of VSDI in the di-synaptic hippocampal pathway and the recording of field excitatory postsynaptic potential (fEPSP) recordings, hippocampal slices of all genotypes (WT, hetNR2B, mAPP and hetNR2B/mAPP) at an age of 13-18 months were tested for spatiotemporal activity propagation and LTP induction. Surprisingly, CA1-LTP induced by high frequency stimulation (HFS; 100Hz / 1s) was not different in all genotypes. In the presence of Aβ1-42 (50nM) potentiation of fEPSP was reduced in WT and hetNR2B/mAPP mice, whereas LTP in mAPP and hetNR2B were not affected. For VSDI a fast depolarization signal (FDS) was evoked in the granule cell layer and propagation was analysed in hippocampal CA3 and CA1 region before and after theta stimulation (θ-stim; 100 pulses / 5Hz). Initial slope and maximal intensity were increased in CA3 and CA1 of mice carrying mAPP. In hetNR2B/mAPP and NR2B, however, these parameters were similar to WT mice indicating a reversal effect of the attenuated NR2B expression on the AD mouse model. Furthermore, in mAPP mice θ-stim produced an epileptiform activity reflected in a pronounced prolongation of the FDS compared to the other genotypes. In summary, the induction of a forebrain specific conditional hetNR2B mutation in the mAPP transgenic restores the pathophysiological changes on hippocampal synaptic plasticity in a mouse model of AD. Thus, these results provide further evidence for the involvement of the glutamatergic system in AD pathology and emphasize the NR2B subunit as a potential target for AD treatment.

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Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.08/C23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01AG042890

Title: Increased presence of cells co-xpressing Sox2 and NeuN in the dentate gyrus of cognitively-intact individuals with substantial Alzheimer’s Disease neuropathology

Authors: *O. ZOLOCHEVSKA[1], V. GHIRARDI[1], N. BJORKLUND[1], R. WOLTJER[4], M.-A. MICCI[2], G. TAGLIALATELA[3];
[1]Neurosci. and Cell Biol., [2]Anesthesiol., [3]Neurol., UTMB, Galveston, TX; [4]Dept. of Pathology, Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder with characteristic neuropathology of amyloid plaques and neurofibrillary tangles that is associated with severe dementia. However, certain individuals (henceforth referred to as non-demented with AD neuropathology - NDAN) remain cognitively intact despite the presence of substantial plaques and tangle neuropathology consistent with what would be normally associated with fully symptomatic AD. It is currently not known how the NDAN patients escape dementia, and understanding such protective mechanisms would reveal targets for the development of a novel, effective treatment for AD. We hypothesize that increased brain reserve is one key factor concurring to NDAN cognitive resistance due to sustained CNS neurogenesis as compared to demented AD patients. To begin testing our hypothesis, in this study we determine the extent of neurogenesis in the hippocampal dentate gyrus (DG) of NDAN cases in comparison with AD patients and age matched healthy individuals. First, we performed fluorescent immunohistochemistry (IHC) staining of hippocampi with Sox2 and NeuN antibodies. Sox2 is a transcription factor which is necessary to maintain or reestablish multipotency or pluripotency of stem cells. NeuN is a nuclear marker of mature neurons. To our surprise, and at variance with what reported in the rodent brain where expression of Sox2 is distinct from NeuN and restricted to pluripotent neural stem cells, we found extensive co-localization of Sox2 and NeuN in cells of the human DG. We also found that such Sox2+/NeuN+ cells were significantly increased in NDAN when compared to AD and control samples. Both the presence of Sox2+/NeuN+ cells, and increased numbers in NDAN cases was further confirmed by flow cytometry analysis of nuclei isolated from frozen brain tissue and stained with relevant antibodies. In addition, AD samples had most nuclei expressing NeuN alone with a significant reduction of nuclei expressing Sox2 alone. Given that simultaneous expression of Sox2 and NeuN may reflect differentiating stem cells or newly formed neurons, these data suggests that there is a positive correlation between the increased amount of neurogenesis in hippocampi of NDAN patients and resistance to cognitive impairment as compared to demented AD or control patients.

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Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.09/C24

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Elucidating the Cognitive Deficits associated with Doxorubicin treatment

Authors: *A. H. ALHOWAIL[1], S. BHATTACHARYA[2], D. BHATTACHARYA[2], J. BLOEMER[2], M. DHANASEKARAN[2], B. SMITH[2], R. ARNOLD[2], V. SUPPIRAMANIAM[2]; [1]Pharmacal Sci., [2]Auburn Univ., Auburn, AL

Abstract: Chemotherapeutic drugs are effective in the treatment of various types of tumors; however, they cause secondary effects including cognitive impairment, also known as "chemobrain" or "chemofog", referring to a phenomenon in which cancer survivors exhibit cognitive impairment following chemotherapy treatment. Chemobrain is observed in more than 75% of cancer patients exposed to chemotherapy, and persistent in 17-34% of cancer survivors. Although the mechanism of cognitive dysfunction is unknown for most of these drugs, some chemotherapeutic agents may trigger cognitive impairment by accessing the brain via the blood-brain barrier (BBB). In our study, six weeks old nude mice were treated with 5mg/kg doxorubicin for four weeks (4 treatments) and the brains were isolated and hippocampi removed. Electrical recordings from acutely isolated hippocampi reveled that long-term potentiation (LTP) was significantly decreased in doxorubicin treated animals compared to controls. Single channel recordings of synaptosomes isolated from animals treated with doxorubicin showed a decrease in the conductance and probability of openings of AMPA-glutamate receptors. Western blot analysis of hippocampal synaptosomes indicated reduction in GluR1/PSD-95 expression and increased GluR2 expression compared to control. Our data suggest that altered expression and function of synaptic AMPA receptors resulted in impaired synaptic deficits contributing to memory impairment associated with doxorubicin treatment.

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Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

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Program#/Poster#: 690.10/C25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Corporation

Solo Non-Profit Research Ltd

Title: Memory improvement in aging community dwelling seniors: A single-blind study of two antioxidant supplements

Authors: *W. K. SUMMERS[1,2], R. L. MARTIN[1], Y. LIU[2], G. M. MARSH[2];
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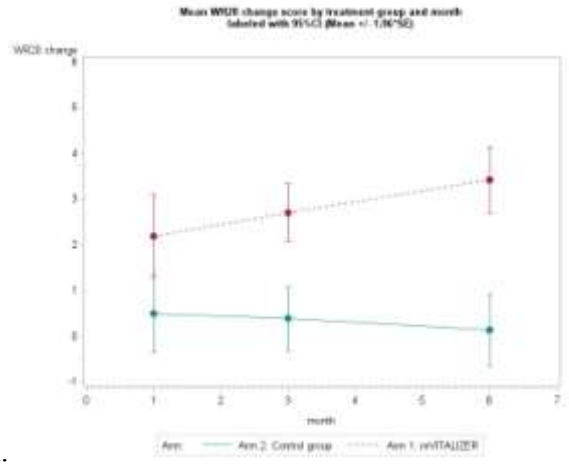
Abstract: Working memory is immediate recall memory, while declarative memory involves either personal experience or factual information. Numerous animal studies, show antioxidants improve memory performance. Human studies with simple antioxidants do not seem to improve memory performance. Use of complex antioxidant mixtures in some human studies have show promise to improve memory capacity. In this study a complex high potency antioxidant blend (Memory reVITALIZER, mR) is compared to a popular one-a-day multivitamin (ODMv) over a 6 month period. 63 community dwelling subjects between 50-75 years old were randomly assigned to one of the two bio-supplements. Memory testing was done at baseline, 1, 3, and 6 months. The 100 item Names-Learning-Test (NLT100) measures competency of the superior temporal lobe connections to other areas of the brain. The 20 Word Recall Test (20WRT) putatively assesses hippocampal competency. 30 mR and 33 ODMv subjects completed the six month trial. The two groups were similar relative to the demographic variables considered. Statistical analysis was via generalized estimating equation to model change score analysis. The high antioxidant mR group showed highly statistically significant improvement over baseline for NLT100 ($p<0.0001$) and 20WRT ($p<0.001$). The improvement in mR group was evident by 1 month. The improvement in NLT100 was static, but the 20WRT showed continued improvement over 6 months. There were no significant improvements in memory testing of ODMv subjects. We conclude that a 35 component potent antioxidant administered over 6 months does improve memory in normal community dwelling humans aged 50-75, beginning with the 1st month.



Disclosures: W.K. Summers: A. Employment/Salary (full or part-time):; Alzheimer's Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alzheimer's Corporation. **R.L.**

Martin: A. Employment/Salary (full or part-time):; Alzheimer's Corporation. **Y. Liu:** None. **G.M. Marsh:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.11/C26

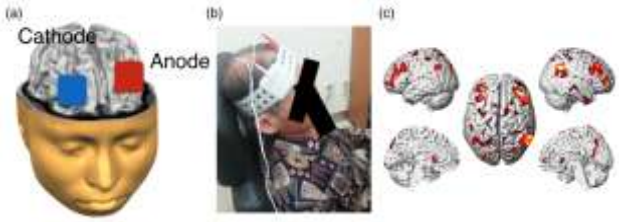
Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Three weeks of transcranial direct current stimulation increased glucose metabolism of mild cognitive impairment

Authors: *J.-C. KIM[1], Y. SHIN[1], H. LEE[1,2], K. YUN[1,3], I.-U. SONG[4], Y.-A. CHUNG[5]; [1]Ybrain Res. Inst., Ybrain Res. Inst., Seoul, Korea, Republic of; [2]Korea Advanced Inst. of Sci. and Technol. (KAIST), Daejeon, Korea, Republic of; [3]Caltech, Pasadena, CA; [4]Departments of Neurol., [5]Departments of Radiology, Col. of Medicine, The Catholic Univ., Seoul, Korea, Republic of

Abstract: Introduction Transcranial direct current stimulation (tDCS) is a method for noninvasively stimulating specific cortical regions of the brain with a mild (<2mA) and persistent direct current. TDCS has been previously applied for the study and treatment of both mild cognitive impairment (MCI) and Alzheimer's disease (AD). Recently, limited imaging techniques were used to investigate the effectiveness of tDCS. In this study, positron emission tomography (PET) imaging was performed to understand glucodynamic changes in the brain on the patients with MCI after sessions of the stimulation. Methods Twenty patients with MCI participated in this experiment. They were randomized to receive active- and sham-tDCS. There were a total of nine 30-minute tDCS sessions for each patient; they received the stimulation three times a week for three weeks. The anode and cathode for tDCS were placed on the left and right dorsolateral prefrontal cortex (DLPFC), respectively. PET imaging was performed before any stimulation and also after the nine sessions to give a visual comparison. The PET images were interpolated to 47 slices, registered, transformed into the coordinates of a standard brain atlas, and smoothed to 8 mm in the x, y and z planes by using statistical parametric mapping (SPM8). At the end of each session, patients were interviewed regarding any pain or discomfort felt during the stimulation. Results Glucose metabolism was increased in multiple brain areas, including visual, somatosensory and other cortical regions, ($n=4$, $p<0.05$, uncorrected for paired sample t-test) after the nine sessions of the stimulation. The glucose metabolism in the prefrontal

cortex was improved the most. Conclusion We performed the DC stimulation and PET imaging on the patients with MCI. We found that overall brain activity was significantly enhanced; the prefrontal region of the brain specifically saw the most increased activity after the nine sessions of stimulation. To the best of our knowledge, this is the first attempt made at investigating the efficacy of tDCS on patients with MCI, via PET



imaging

Disclosures: J. Kim: None. **Y. Shin:** None. **H. Lee:** None. **K. Yun:** None. **I. Song:** None. **Y. Chung:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.12/C27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: by a grant (kiom-2010-2) from the Inter-Institutional Collaboration Research Program provided by the Korea Research Council of Fundamental Science & Technology

Title: Fructus mume improved memory impairments and reduced neuroinflammation in 5xFAD transgenic mice

Authors: J.- . PARK[1], J. MA[1], W. JEON[2], *J.-S. HAN[1];
[1]Biol. Sci., Konkuk Univ., Seoul, Korea, Republic of; [2]Korea Inst. of Oriental Med., Daejeon, Korea, Republic of

Abstract: Fructus mume (F. mume) has been reported to have anti-inflammatory effects. The previous study has demonstrated that F. mume extracts improved cognitive deficit and alterations of mitogen-activated protein kinase (MAPK) and NF-kappa B signaling induced by chronic cerebral hypoperfusion. The present experiment was conducted to examine effects of F. mume extracts on memory-improving effects in Alzheimer’s disease (AD) using 5xFAD mice with five familial AD mutations. It has been reported that 5xFAD mice exclusively generate amyloid beta-

42, which is accumulated in massive brain regions. Daily administration of F. mume extracts was started at 3 months of age and continued for 80 days. The status of hippocampus-dependent memory was evaluated in Morris water maze task, novel object/location recognition test, and contextual fear conditioning at the age of 6 months. In the behavioral tasks, 5xFAD mice that were treated with vehicle showed impairments of hippocampus-dependent memory compared with those of non-Tg littermates. However, F. mume-treated 5xFAD mice performed improvement of spatial memory in acquisition training in Morris water maze task. Also, they showed improvements of location recognition memory and contextual fear memory. To examine effects of F. mume on alterations in inflammatory response including expression of microglia and astrocyte, western blot analysis and immunohistochemistry were conducted. F. mume treatment reduced expression levels of these glia cells. These results indicate that F. mume might have memory-improving effect on AD through suppressing the inflammation.

Disclosures: J. Park: None. **J. Han:** None. **J. Ma:** None. **W. Jeon:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACYT CB2012/178841

CONACYT CB2012/155242

DGAPA-UNAM IN201613

DGAPA-UNAM IN209413

Title: Impaired spatial memory retrieval is related to dysfunctional Arc protein expression in the CA3 region of a mouse model of Alzheimer’s disease

Authors: J.-P. MORIN[1], G. VELAZQUEZ-CAMPOS[1], A. AGUILAR-VAZQUEZ[1], A. PINEDO-VARGAS[1], C. PEREZ-CRUZ[2], F. BERMUDEZ-RATTONI[3], *S. DIAZ-CINTRA[1]; [1]UNAM Campus Juriquilla, Queretaro, Mexico; [2]FARMACOLOGIA, CINVESTAV, MEXICO, Mexico; [3]Inst. of Cell. Physiology, UNAM, Mexico, Mexico

Abstract: Dysfunction of synaptic communication in hippocampal networks has been suggested as one of the neuropathological hallmarks of the early stages of Alzheimer's Disease (AD). In numerous AD mice models, disrupted levels of Activity-regulated cytoskeletal associated protein (Arc) an immediate early gene (IEG) product that plays a central role in synaptic plasticity, were observed in memory-relevant forebrain regions (Kerrigan & Randall, 2013). As other IEG products, Arc expression patterns in hippocampal networks has been extensively used as a marker of memory-relevant neuronal activity. However, no study to date has examined whether Arc protein expression is altered in 3xTg-AD mice, a milder model of AD. Here, we used immunohistochemistry and confocal microscopy to evaluate Arc protein levels in basal conditions and after brief reactivation of a spatial memory task. Because of its known rapid activation and role in one trial, learning, our analysis focused on the hippocampal CA3 region. Our data show that the percentage of Arc protein expressing cells under basal conditions is higher in the 3xTg-AD mice than in their age-matched controls. Furthermore, although Morris Water Maze retrieval (test phase) induced a steep increase in the percentage of cells expressing Arc protein in the control animals, no such increase was observed in the 3xTg-AD group. Strikingly, subsequent analysis showed a highly significant positive correlation between the proportion of Arc expressing cells and memory precision when animals of both groups were taken into account. These data may suggest that in 3xTg-AD mice, CA3 networks are saturated under basal conditions thus impeding further demands on the memory-stabilizing process.
Kerrigan, T. L., & Randall, A. D. (2013). A new player in the “synaptopathy” of Alzheimer’s disease - arc/arg 3.1. *Frontiers in Neurology*, *4*(February), 9.

Disclosures: J. Morin: None. **G. Velazquez-Campos:** None. **A. Aguilar-Vazquez:** None. **A. Pinedo-Vargas:** None. **S. Diaz-Cintra:** None. **F. Bermudez-Rattoni:** None. **C. Perez-Cruz:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.14/C29

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Dynamics of cognitive-enhancing erk -pparγ protein complexes

Authors: *D. CORTEZ[1], T. J. URBAN[2], A. L. DIMET[2], J. B. JAHRLING[2], L. DENNER[2], K. T. DINELEY[2];
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Abstract: The MAPK/ERK pathway is crucial for hippocampus-dependent learning and memory that is dysregulated in the earliest phases of Alzheimer's disease (AD). In neurons, activation of ERK promotes interactions with downstream effectors necessary for new gene transcription and protein synthesis necessary for memory consolidation. Phosphorylated, and therefore active, ERK recognizes substrates through consensus D and DEF binding motifs. We recently discovered that PPARγ (peroxisome proliferator activated receptor gamma) harbors these putative binding motifs. Further, pERK-PPARγ complex formation correlates with cognitive reserve in human AD temporal cortex and is necessary for hippocampus-dependent memory consolidation in the Tg2576 AD mouse model. Here we determined the spatio-temporal dynamics and compositional identification of complex components using animal and cellular models such as iPSC-derived neurons. Translational endeavors include small molecule screening to identify compounds that enhance complex formation *in vitro* and *in vivo* as well as improving cognition in AD animal models.

Disclosures: D. Cortez: None. **T.J. Urban:** None. **A.L. Dimet:** None. **J.B. Jahrling:** None. **L. Denner:** None. **K.T. Dineley:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

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Program#/Poster#: 690.15/C30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG037481

NIH R01AG037919

NIH K01AG044490

Alzheimer's Association NIRG

Title: Abca1 deficiency significantly affects neurite morphology and cognitive function in mice

Authors: *A. Y. CARTER[1], N. F. FITZ[1], V. M. TAPIAS[2,3], E. L. CASTRANIO[1], I. LEFTEROV[1], R. KOLDAMOVA[1];
[1]Envrn. and Occup. Hlth., [2]Neurol., [3]Pittsburgh Inst. for Neurodegenerative Dis., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: ATP-binding cassette transporter A1 (ABCA1) mediates cholesterol efflux to lipid-free apolipoproteins and regulates the generation of high density lipoproteins (HDL). ABCA1 deficiency in brain affects apoE lipidation and stability leading to a significant decrease of apoE-containing lipoproteins. We have previously shown that lack of Abca1 significantly increases amyloid deposition and cognitive deficits in mice expressing human amyloid β precursor protein. However, the normal physiological role of ABCA1 in the brain is currently unknown. Our hypothesis is that in CNS ABCA1 maintains cholesterol transport from glial cells to neurons. In the brain cholesterol plays an important role during myelination, neurite outgrowth and repair, and synaptic vesicle formation. The goal of this study was to examine neurite architecture of pyramidal hippocampal neurons in Abca1ko mice and how this correlates to cognitive deficits. Our data demonstrate that Abca1ko mice had a significantly impaired performance during the probe trial of Morris water maze but the acquisition phase was not affected. Confocal scanning laser microscopy with complementary 3D image analysis of medial hippocampal sections revealed a significant decrease in neurite length, number of neurite segments, and number of branches in the CA1 region of Abca1KO mice when compared to WT mice; however, no changes were observed in region CA2. These results suggest the importance of disruption of Abca1 for neurite degeneration in the hippocampus, which is region-specific.

Disclosures: A.Y. Carter: None. **N.F. Fitz:** None. **V.M. Tapias:** None. **E.L. Castranio:** None. **I. Lefterov:** None. **R. Koldamova:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

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Program#/Poster#: 690.16/C31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF IOS 08-43175

NSF IOS 13-18490

NIA P30 AG034464

Alzhiemer's Association

NIH HD007333

Title: Memory deficits in Alzheimer's disease model mice coincide with appearance of amyloid plaques and are preceded by insensitivity to glucose enhancement of memory

Authors: *L. A. NEWMAN, D. L. KOROL, P. E. GOLD;
Biol., Syracuse Univ., Syracuse, NY

Abstract: In both rats and humans, glucose administration enhances memory across multiple cognitive domains. In rats, intrahippocampal injections of glucose reverse age-related impairments for inhibitory avoidance memory and spatial working memory. The present study analyzed the ability of glucose to enhance spatial working memory in a transgenic mouse model of Alzheimer's disease (B6C3-Tg(APPswe,PSEN1dE9)Dbo/J). Fifteen min prior to spontaneous alternation testing, microinjections of glucose (10, 20 or 40 nmol) or saline were given into the dorsal hippocampus of transgenic or wild type mice at 3, 6, 12, or 18-24 months of age. Overall, wild type mice exhibited better spatial working memory than did transgenic mice with impairments evident in the transgenic mice at 6 and 18-24 months of age. Glucose enhanced memory in 3-mo-old wild type mice but not in the transgenic mice. Congo Red staining revealed no significant amyloid plaque development in the 3-mo-old transgenic mice but significant plaque formation at later ages; plaques were not seen in the wild type mice. These findings suggest that onset of memory impairments and amyloid plaque formation may be co-morbid in the transgenic mice. Interestingly, the 3-mo-old transgenic mice, which showed no impairment of memory, were not susceptible to enhancement of memory by glucose. Glucose was, however, effective at enhancing memory in 3-mo-old wild type mice. Our previous findings in rats suggest that glucose may act through glycogenolysis in astrocytes rather than directly on neurons to enhance memory. Together with the present results showing a failure to see enhancement of memory in the transgenic mice, it is possible that poor utilization of glucose by astrocytes and, therefore, poor bioenergetics available to support memory processing may contribute to and be an early marker of the development of later impairments in memory.

Disclosures: L.A. Newman: None. **D.L. Korol:** None. **P.E. Gold:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.17/C32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR grant MOP-126001

Heart and Stroke Foundation of Québec

FRQS-NSFC collaborative grant

Title: Simvastatin rescues cognitive and cerebrovascular deficits induced by high cholesterol diet in transgenic TGF mice

Authors: X.-K. TONG, *E. HAMEL;
Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Introduction: Transgenic mice overexpressing transforming growth factor-β1 (TGF mice) display a cerebrovascular pathology characterized by vascular fibrosis, cerebrovascular remodeling, and string vessel pathology. These alterations result in impaired cerebrovascular reactivity, chronic cerebral hypoperfusion, and neurovascular uncoupling. However, TGF mice do not display cognitive deficits or show only minor changes in their performance with increasing age. Cardiovascular diseases being a major risk factor for cognitive impairment and, particularly, vascular dementia, we investigated the impact of a high cholesterol diet (HCD) on cerebrovascular and cognitive function in adult and aged TGF mice. In adult mice, we also tested the benefit of simvastastin (SV), an anti-cholesterol drug with pleiotropic effects. Methods: TGF mice were fed a HCD (2% cholesterol and 0.5% cholic acid) for 3 months, and tested at 6 (adult) or 12 (aged) months. An additional cohort of adult mice was treated with HCD with or without SV (40 mg/kg/day) for 3 months. HCD and SV effects were evaluated using ANOVA, and a $p<0.05$ was considered significant. Results: HCD significantly increased blood cholesterol levels in both wild-type (WT) and TGF mice, and SV treatment exerted no reducing effect. In adult and aged TGF mice, HCD worsened the deficits in dilatory function measured with acetylcholine or calcitonin gene-related peptide, with a more pronounced effect in aged mice. SV treatment fully restored dilatory responses, acting in part through normalization of KATP and TRPV4 channel function. The impaired neurovascular coupling response to whisker stimulation in adult and aged TGF mice was not altered by HCD whereas SV treatment normalized this response. When tested for spatial learning and memory in the Morris water maze, neither adult nor aged TGF mice display any significant deficits although aged TGF mice were slightly slower than WT controls in learning the location of the hidden platform. TGF mice of both age groups fed a HCD developed cognitive impairments, and SV treatment totally rescued these deficits in adult TGF mice. Conclusions: The results show that HCD significantly exacerbates cerebrovascular dysfunction and precipitates cognitive decline in TGF mice, but not in WT mice, suggesting that an underlying vascular pathology facilitates the expression of cognitive failure. SV, independent of its cholesterol lowering effects, countered all deleterious effects of HCD on vascular and neuronal function. These results suggest that SV may bear promise in preventing or delaying cognitive failure related to vascular dementia.

Disclosures: **X. Tong:** None. **E. Hamel:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.18/C33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: University of Hartford Neuroscience Graduate Program

Title: Effects of ketogenic diets on cognition and development of alzheimer's disease (ad) pathology in an ad mouse model

Authors: ***J. P. HARNEY**[1], J. GRIZZANTI[2], M. BARRY[2], K. MCCARTHY[2], D. BUTLER[2]; [1]Univ. Hartford, WEST HARTFORD, CT; [2]Univ. of Hartford, West Hartford, CT

Abstract: For decades the ketogenic diet (KD), a high fat, low carbohydrate and protein diet, has been an acceptable and moderately effective treatment of intractable epilepsy in children. In recent years therapeutic use of the diet has been extended to neural degeneration produced by a variety of other insults including cerebral ischemia, traumatic brain injury, neuro- and excito-toxins and in a transgenic mouse model of Huntington's disease. Recent studies describing an associative relationship between type 2 diabetes and Alzheimer's disease (AD) suggests that altered brain energy metabolism may impact development of cognitive impairment and neuropathology in AD. Thus, the focus of the present study was to determine if KDs fed to AD mice would alter the development of cognitive decline and AD-associated neuropathology. Four month old Tg(APPSwe,tau301L) mice and control mixed background (B6129SF2/J) wildtypes (WT) (n=40) were randomly assigned to either a standard rodent chow (RC) diet or one of 2 KDs (KD8%, 8% protein; KD14%, 14% protein), and housed individually. After twelve weeks on the diets mice were tested for learning and memory using the Morris water maze. Weekly weights showed steady modest weight gain for all groups. Bi-weekly blood ketones were elevated ($p<0.05$) in all mice on KDs with KD8% displaying the greatest ketosis. AD mice displayed diminished initial learning compared to WT, but KD14% and RC fed-AD mice improved the rate ($P<0.05$) of learning compared to KD8% fed mice. Interestingly, KD8%-fed mice performed best on 24h memory test compared ($P<0.05$) to KD14% and RC-fed mice. Collectively the results suggest that varying the protein content of KDs may impact their effectiveness when used therapeutically for the treatment of neurodegenerative disorders like AD. AD-associated

neuropathology currently being assessed biochemically will be evaluated with behavioral outcomes.

Disclosures: J.P. Harney: None. **J. Grizzanti:** None. **M. Barry:** None. **D. Butler:** None. **K. McCarthy:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.19/C34

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Leukotriene receptor inhibition promotes neurogenesis, decreases neuroinflammation and improves cognition in aging

Authors: *J. MARSCHALLINGER[1], S. COUILLARD-DESPRES[2], B. KLEIN[1], C. SCHMUCKERMAIR[3], S. ILLES[1], R. CORAS[4], I. BLUEMCKE[4], N. SINGEWALD[3], L. AIGNER[1];
[1]Inst. for Mol. Regenerative Med., [2]Exptl. Neuroregeneration, PMU Salzburg, Salzburg, Austria; [3]Pharmacol. and Toxicology, Leopold-Franzens-University of Innsbruck, Innsbruck, Austria; [4]Neuropathology, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany

Abstract: New neurons are generated throughout life in the adult brain. With advancing age, the rate of adult neurogenesis dramatically declines, and this reduced formation of new neurons strongly correlates with the occurrence of cognitive decline during aging and in neurodegenerative diseases. The mechanisms responsible for the decline of neurogenesis and for the decrease of cognitive skills in the elderly are still not fully understood; however, neuroinflammatory processes seem to play a prominent role. The lipid inflammatory mediators leukotrienes and the associated 5-LOX signaling pathway very likely contribute to the development of dementia. Concentrations of these molecules are specifically elevated within the hippocampal neurogenic region in the aged rodent brain, indicating a negative involvement of leukotriene signaling on adult neurogenesis. In the present study, we analyze the impact of a 6 weeks oral treatment with the leukotriene receptor antagonist montelukast on neuroinflammation and on adult neurogenesis *in vivo* as well as possible effects on behavior in young (4 months) and aged (20 months) rats. Standardized behavior tests (Open Field, Elevated Plus Maze, Forced Swim Test, Morris Water maze) and histological analyses of the hippocampus, i.e. neural progenitor proliferation, differentiation, cell survival, neuroinflammation and neuronal synaptic

density were assessed. Our data revealed that inhibition of the leukotriene signalling pathway promotes neurogenesis and decreases neuroinflammatory characteristics specifically in old rats. Most intriguingly, montelukast significantly improves learning and memory skills in old rats to a level comparable with healthy young rats. Thus, inhibition of the leukotriene signaling pathway might provide a promising approach to compensate the cognitive decline during aging and in neurodegenerative diseases.

Disclosures: J. Marschallinger: None. **C. Schmuckermair:** None. **R. Coras:** None. **B. Klein:** None. **S. Illes:** None. **L. Aigner:** None. **N. Singewald:** None. **I. Bluemcke:** None. **S. Couillard-Despres:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.20/C35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: kiom-2010-2

Title: Rats with chronic bilateral common carotid artery occlusion showed impairments of intra-dimensional shift in attentional set shifting task

Authors: *B.-R. CHOI[1], D.-H. KIM[1], W. JEON[2], J.-S. HAN[1];
[1]biology, Konkuk Univ., Seoul, Korea, Republic of; [2]creative research laboratory, Korea institute of oriental medicine, Daejeon, Korea, Republic of

Abstract: Persistent insufficient cerebral blood leads to vascular dementia (VaD), one of the most common neurodegenerative diseases. Animal model using chronic bilateral common carotid artery occlusion (BCCAo) has been used for the mechanism study and therapeutic developments for VaD. A line of study has reported that rats with chronic BCCAo show neuronal degeneration, increased inflammation, and cognitive impairments. But no study has conducted to examine attentional dysfunction in which broadly observed in VaD patients. Therefore, the present study was conducted to examine attentional function in rats with BCCAo, using attentional-set shifting task. During attentional-set shifting task, a rat had to choose one food-baited bowl between two bowls. Attentional-set shifting task is composed with consecutive sessions including simple discrimination, compound discrimination, intra-dimensional shifting, extra-dimensional shifting, and reversal. The BCCAo rats were impaired to perform the intra-

dimensional shifting in attentional set shifting task compared to sham-operated control rats. According to the previous studies, cingulate cortex and medial prefrontal cortex play a role in performing intra-dimensional set shifting and extra-dimensional set shifting, respectively. We examined chronic BCCAo-induced neuronal alterations and neuro-inflammatory responses in cingulate cortex and prefrontal cortex using immunohistochemistry. In cingulate cortex and prefrontal cortex, a number of OX-6 or Iba-1 positive microglia were increased in BCCAo rats compared with sham-operated control rats. And these were also increased in white matter of BCCAo rat. These results indicate that dysfunction of prefrontal and cingulate cortex and damages of projection to other brain areas in BCCAo rats could be contribute to attentional impairments.These results would provide an understanding of impairments of cognitive function and its mechanism in VaD. This study was supported by a grant (kiom-2010-2) from the Inter-Institutional Collaboration Research Program provided by the Korea Research Council of Fundamental Science & Technology (KRCF), Korea.

Disclosures: **B. Choi:** None. **D. Kim:** None. **W. Jeon:** None. **J. Han:** None.

Poster

690. Alzheimer's Disease: Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 690.21/C36

Topic: F.01. Human Cognition and Behavior

Title: Older adults with Alzheimer's disease and normal cognition demonstrate successful learning of an unseen category prototype

Authors: *J. S. PHILLIPS[1], N. MIN[1], P. KOENIG[1], C. MCMILLAN[1], E. E. SMITH[2], M. GROSSMAN[1];
[1]Neurol., Univ. of Pennsylvania, Philadelphia, PA; [2]Psychology, Columbia Univ., New York, NY

Abstract: In acquiring a new category, individuals' encoding of the category depends on the learning task and the features of specific exemplars encountered during learning. We trained healthy older adults (n=41) and individuals with a diagnosis of Alzheimer's disease (AD) or mild cognitive impairment (MCI; combined n=43) to recognize instances of a fictitious animal, the "crutter". Stimuli were images depicting variants of a crutter which could take one of two values on each of 10 anatomical features. Category membership was non-deterministic: learning was evaluated relative to an ideal model in which the likelihood of endorsing a test item as a crutter

was directly proportional to the number of features shared with the prototypical image. We hypothesized that individuals trained on exemplars with a high proportion of the prototype's features (8/9 out of 10) would display better category learning than those trained on exemplars with fewer of the prototype's features (6/7 out of 10). We additionally hypothesized that category learning would depend on whether participants were told that training items belonged to a common semantic category (explicit condition) or were simply instructed to think about the appearance of training items (implicit condition). We predicted elderly controls would learn equally well in implicit and explicit conditions, while the AD/MCI group would learn better in the implicit condition. These hypotheses resulted in between-subjects factors of training feature density (6/7 vs. 8/9), instruction type (explicit vs. implicit), and diagnosis (AD/MCI vs. elderly control). Test items varied in the number of a prototype's features from 0 (antitype) to 10 (prototype). AD/MCI participants demonstrated more difficulty with category learning than elderly controls. However, AD/MCI participants also learned to categorize items in proportion to the number of prototypical features. Both groups benefited from the higher density of prototypical features in the 8/9 condition, although elderly controls appeared to benefit more in this condition than the AD/MCI group. Contrary to hypothesis, AD/MCI participants performed equivalently in explicit and implicit conditions. Among elderly controls, a modest effect of instruction type was observed, with better category discrimination in implicit than explicit conditions. Results demonstrate learning of a category prototype is improved when training materials exhibit high overlap of critical features. Results in the AD group suggest that individuals with degeneration of the hippocampus and medial temporal lobes may be capable of category learning, even when stimuli vary on a large number of features.

Disclosures: J.S. Phillips: None. **N. Min:** None. **P. Koenig:** None. **C. McMillan:** None. **E.E. Smith:** None. **M. Grossman:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.01/C37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG000259

Title: Cholinotrophic and galaninergic profiles in the domestic pig forebrain (Sus scrofa domesticus)

Authors: *L. MAHADY, E. J. MUFSON, S. E. PEREZ;
Neurosci., Rush Univ., Chicago, IL

Abstract: The distribution of cholinergic neurons within the basal forebrain has been evaluated in numerous mammalian species since they play a key role in learning, memory and attentional behaviors. In most mammals these neurons contain choline acetyltransferase (ChAT), the synthesizing enzyme for acetylcholine (Ach), the low affinity $p75^{NTR}$ receptor for nerve growth factor and the Ach neuromodulator, galanin. However, there are no studies of the cholinergic system in the even-toed hoofed mammal domestic pig (*Sus scrofa domesticus*), which is often used in preclinical studies. Therefore, we examined the distribution of cholinergic related systems in the domestic pig forebrain. Following a 4% paraformaldehyde transcardial perfusion, brains were harvested from 4 month old female pigs and sections were immunohistochemically stained with antibodies against ChAT (Millipore 1:1,000), the low-affinity neurotrophin receptor $p75^{NTR}$, (Millipore, 1:3,000) and (GAL) (gift from Dr. E. Theodorsson, Sweden, 1:1,000). Bright field microscopy revealed small, multipolar ChAT immunoreactive (-ir) neurons within the lateral and medial septal nuclei, vertical and horizontal limbs of the diagonal band of Broca, and larger multipolar perikarya in the nucleus basalis of Meynert. In addition, ChAT-ir multipolar and bipolar neurons were found in the striatum and the cortex, respectively, but not in the hippocampus. Cholinergic positive neurons were also observed in the olfactory tubercle and medial habenula. $p75^{NTR}$-ir neurons were found in the medial septum, diagonal band of Broca, nucleus basalis of Meynert, and the olfactory tubercle but not in the cortex or hippocampus. GAL immunoreactive neurons were not seen in the cholinergic subfields. Numerous ChAT and $p75^{NTR}$-ir fibers were seen throughout the cortex and hippocampus compared to GAL-ir fibers, which were more pronounced in the lateral septum and the bed nucleus of the stria terminalis. These findings indicate that the distributions of cholinotrophic and galaninergic profiles within the domestic pig forebrain are to a great extent similar to those seen in other phylogenetically related mammals.

Disclosures: L. Mahady: None. **E.J. Mufson:** None. **S.E. Perez:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

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Program#/Poster#: 691.02/C38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Novo Nordisk

Title: The GLP-1 analog liraglutide improves memory function and prevents hippocampal neuronal loss in a senescence-accelerated mouse prone (SAMP8) model of Alzheimer's disease

Authors: *H. H. HANSEN[1], P. BARKHOLT[1], K. FABRICIUS[1], M. NIEHOFF[2], J. E. MORLEY[2,3], J. JELSING[1], C. PYKE[4], L. B. KNUDSEN[4], S. A. FARR[2,5], N. VRANG[1]; [1]Gubra, Hoersholm, Denmark; [2]St. Louis Univ. Div. of Geriatrics, St. Louis Univ., St. Louis, MO; [3]Div. of Endocrinol., St. Louis Univ. Sch. of Med., St. Louis, MO; [4]Diabetes Res., Novo Nordisk, Maaloev, Denmark; [5]Res. and Develop., Veterans Affairs Med. Ctr., St. Louis, MO

Abstract: Numerous reports have indicated that glucagon-like peptide 1 (GLP-1) receptor agonists possess neurotrophic and neuroprotective effects in rodent models of Alzheimer's disease (AD). Liraglutide, a once-daily GLP-1 analog, has potent incretin effects and is currently used in the management of type 2 diabetes. In the current study we investigated the potential pro-cognitive and neuroprotective effects of liraglutide treatment for 5 months in senescence-accelerated mouse prone (SAMP8) mice, a spontaneous animal model of pathological aging with characteristics of neurobehavioral and neuropathological impairments observed in early-stage AD. Six-month old SAMP8 mice received liraglutide (100 or 500 µg/kg/day, s.c.) or vehicle. Vehicle-dosed 50% backcrossed as well as non-dosed young (four-month old) SAMP8 mice were used as additional control groups. Experimental groups sizes were at least n=10 mice per group. Ten-month old vehicle-dosed SAMP8 mice showed significant deficits in learning and memory retention in an active-avoidance T-maze. Also, these mice displayed a mild loss of hippocampal CA1 neurons with no concurrent immunohistological signatures of beta-amyloid and hyperphosphorylated Tau, indicating the onset of cognitive deficits prior to deposition of amyloid plaques and neurofibrillary tangles in this AD model. Interestingly, liraglutide markedly increased memory retention performance in an active-avoidance T-maze. Memory consolidation in liraglutide-treated mice was almost at the level determined in backcrossed and young SAMP8 mice, respectively. Also, liraglutide-treated SAMP8 mice showed a significantly higher hippocampal CA1 neuronal number and density as compared to ten-month old vehicle-treated mice. In conclusion, liraglutide delayed or, alternatively, halted the age-associated progressive decline in learning and memory function associated with hippocampal neuronal loss in a spontaneous mouse model of AD.

Disclosures: **H.H. Hansen:** None. **P. Barkholt:** None. **K. Fabricius:** None. **M. Niehoff:** None. **J.E. Morley:** None. **J. Jelsing:** None. **C. Pyke:** None. **L.B. Knudsen:** None. **S.A. Farr:** None. **N. Vrang:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.03/C39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MINECO-FEDER BFU2011-22740

Title: GirK channel, a new target in early stages of Alzheimer Disease

Authors: M. O. NAVA-MESA[1], L. JIMENEZ-DIAZ[2], J. YAJEYA[4], *J. NAVARRO-LOPEZ[3]; [1]Neurosci. Res. Group (Neuros), Univ. of Rosario, Bogotá, Colombia; [2]Neurophysiol. & Behaviour Lab., [3]Univ. of Castilla-La Mancha, Ciudad Real, Spain; [4]Physiol. & Pharmacol., Univ. of Salamanca, Salamanca, Spain

Abstract: Last evidences suggest that, in Alzheimer's disease (AD) early stage, Amyloid-β (Aβ) peptide induces an imbalance between excitatory and inhibitory neurotransmission systems resulting in the functional impairment of neural networks. Such alterations are particularly important in the septohippocampal system where learning and memory processes take place depending on accurate oscillatory activity tuned at fimbria-CA3 synapse. Here, the acute effects of Aβ on CA3 pyramidal neurons and their synaptic activation from septal part of the fimbria were studied in rats. A triphasic postsynaptic response defined by an excitatory potential (EPSP) followed by both early and late inhibitory potentials (IPSP) was evoked. The EPSP was glutamatergic acting on ionotropic receptors. The early IPSP was blocked by GABAA antagonists whereas the late IPSP was removed by GABAB antagonists. Aβ perfusion induced recorded cells to depolarize, increase their input resistance and decrease the late IPSP. Aβ action mechanism was localized at postsynaptic level and most likely linked to GABAB-related ion channels conductance decrease. In addition, it was found that the specific pharmacological modulation of the GABAB receptor effector, G-protein-coupled inward rectifier potassium (GirK) channels, mimicked all Aβ effects previously described. Thus, our findings suggest that Aβ, altering GirK channels conductance in CA3 pyramidal neurons, might have a key role in the septohippocampal activity dysfunction observed in AD.

Disclosures: M.O. Nava-Mesa: None. **L. Jimenez-Diaz:** None. **J. Navarro-Lopez:** None. **J. Yajeya:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MINECO-FEDER BFU2011-22740

MHE 2011-00118

RYC-2009-03827

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BFU 2009-07341

Title: GirK and KNCQ channels are targets for amyloid-β in the rodent hippocampus

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Abstract: Early stage of Alzheimer Disease (AD) is characterized by a synaptic dysfunction state induced by soluble amyloid-β peptide (Aβ) which impairs the balance between excitatory and inhibitory neurotransmission in learning and memory related regions such as the hippocampus. Recently, several mechanisms of loss-of-function of sodium or potassium channels which control neuronal excitability have been proposed to contribute to the alteration in AD of hippocampal inhibitory neurotransmission, and subsequent network hyperactivity and hypersynchrony. Because the hippocampus has been related to cognitive deficits in AD, the main aim of the present study was to determine the putative Aβ molecular targets in this region, as well as behavioral changes induced by Aβ intracerebroventricular injection in behaving mice. Quantitative Real-Time PCR (qPCR) was performed to analyze the expression pattern of 17 genes related with excitatory and inhibitory neurotransmission in compliance with the MIQE (Minimum Information for Publication of qPCR Experiments) guidelines. In order to make a

correct interpretation of gene expression data, three putative reference genes, Actb, Gapdh and Ppia were investigated and ranked according to their expression stability by BestKeeper and NormFinder algorithms. To study the effects of hippocampal Aβ injection in behaving mice, object recognition and open field tests were performed. Our results indicate that Aβ modulates the expression of GirK and KCNQ potassium channels in the hippocampus, which could contribute to the excitatory/inhibitory hippocampal neurotransmission imbalance that causes the aberrant network activity and the early cognitive impairment found in AD models. In addition, preliminary data collected from behavioral experiments showed differences in both tests for Aβ-treated animals making our behavioral model an interesting tool for the study of GirK and KCNQ roles in early AD.

Disclosures: J. Mayordomo-Cava: None. **J. Navarro-López:** None. **A. Gruart:** None. **J.M. Delgado-García:** None. **J. Yajeya:** None. **L. Jimenez-Diaz:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.05/C41

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: *In vitro* pharmacological and electrophysiological profile of S 47445, a novel positive allosteric modulator of AMPA type glutamate receptors

Authors: *L. DANOBER[1], J.-Y. THOMAS[1], S. CHALLAL[1], N. ROGEZ[1], K. ALBINET[1], F. IOP[1], N. VILLAIN[1], A. CORDI[2], P. LESTAGE[1];
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Abstract: Positive allosteric modulators of AMPA receptors facilitate AMPA-mediated responses by either reducing the desensitisation of AMPA receptors or by slowing their deactivation. Among them, S 47445 is a novel and selective positive allosteric modulator of glutamate AMPA-type receptors which possesses neuroprotective and cognitive enhancing properties in rodents. The aim of the present study was to investigate the mechanism of action of S 47445 and is effect on neuronal plasticity, by examining its effect on Long-Term Potentiation (LTP) of the synaptic response and on brain-derived neurotrophic factor (BDNF) expression. S 47445 was evaluated *in vitro* on about a hundred receptors, enzymes and channels. The IC50 values in all these assays were greater than 10 µM. S 47445 did not present affinity towards

orthosteric binding sites of AMPA receptors but increased AMPA-mediated depolarization measured by fluorescent membrane potential dyes and an imaging based plate reader, on rat primary brain cell cultures (EC50: 5µM). Similarly, on oocytes injected with either rat cortex or human hippocampal mRNA, S 47445 increased AMPA-evoked current with similar EC50 close to 7 µM (7-8 fold increase of the AMPA response) and did not affect NMDA and kainate-evoked current. In the absence of AMPA, S 47445 had no effect on the holding current of the recorded oocytes. On rat hippocampal slices, S 47445 alone failed to provoke any significant presynaptic noradrenaline release. However, S 47445 dose-dependently (10-300 µM) enhanced AMPA-mediated noradrenaline release. In mature rat primary cortical neurons, S 47445 (100 µM, incubation: 24h) failed to provoke any significant neurotoxicity in comparison with vehicle-treated neurons as assessed by LDH measurements. S 47445 showed neurotrophic properties *in vitro*: S 47445 both increased the expression of BDNF protein when applied alone on rat primary cortical cell cultures (3-100 µM) and was able to stimulate AMPA-induced expression of BDNF protein. *In vivo*, S 47445 (10-30 mg/kg ip) increased both the induction and the maintenance of the LTP of the synaptic response induced by 4 bursts tetanus in the dentate gyrus of the hippocampus in anaesthetized Wistar rats. Taken together, these results indicate that S 47445 via a selective positive allosteric modulation of AMPA receptors enhances neuronal and synaptic plasticity (LTP, BDNF expression), phenomenon that could be of interest for the treatment of various neurodegenerative diseases.

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Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.06/C42

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: *In vivo* pharmacological profile of S 47445, a novel positive allosteric modulator of AMPA type glutamate receptors

Authors: *C. LOUIS[1], L. DANOBER[2], I. CARRIÉ[2], N. DUMAS[2], K. ALBINET[2], K. LLOPIS[2], A. ROGER[2], M.-H. GANDON[2], A. HUGOT[2], M. KRENTNER[2], J.-Y. THOMAS[2], N. ROGEZ[2], M. VANDESQUILLE[2], A. KRAZEM[5], D. BÉRACOCHÉA[5], V. BERTAINA-ANGLADE[6], C. DRIEU LA ROCHELLE[6], C. JUNGES[3], M. BERTRAND[3], S. BILLIALD[3], C.

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Abstract: S 47445 is a novel and selective positive allosteric modulator of glutamate AMPA-type receptors which possesses neurotrophic properties *in vitro* and enhanced synaptic plasticity *in vivo*. The aim of the present study was to characterize *in vivo* pharmacological properties of S47445. Permeability of the Blood-Brain-Barrier was high in rats and mice with partition coefficients (Kp) ranging from 4.3 and 5.8 and brain concentrations of 82 and 333 ng/g, respectively (evaluation 60 min following 3 mg/kg p.o. administration). In agreement with cerebral exposition of the compound, S 47445 showed robust pro-cognitive effects in various models of episodic and spatial working memories in rodents. S 47445 improved the retention of episodic memory in the object recognition test in natural forgetting settings in young rat (0.3, 1 and 3 mg/kg p.o.) and partially counteracted scopolamine-induced amnesia (0.3, 1 and 3 mg/kg p.o.). In mice as well S 47445 improved object recognition performance in natural forgetting settings (1 and 3 mg/kg p.o.) and counteracted alprazolam-induced memory deficits in a delayed spatial discrimination task (3 mg/kg p.o.). Furthermore, S 47445 was able to reverse also age-induced deficits in contextual memory performances in middle-aged mice (0.03, 0.1, 0.3 and 1 mg/kg/d p.o., 9 days of administration). S 47445 both improved spatial working memory both in the spontaneous alternation test on a T-maze in 5 months old mice (0.3 and 1 mg/kg i.p.) and on Y-maze in old 22 months mice (3 mg/kg p.o.). Besides its potent procognitive and neurotrophic action, S 47445 also displayed neuroprotective activity in a model of hippocampal vulnerability in rats (Pulsinelli model, 30 mg/kg i.p., acute). S 47445 did not induce wake-promoting effect as assessed by cortical EEG recordings in freely moving rats (10 and 30 mg/kg i.p., acute) nor modify pentobarbital induced sleep duration in rats (10 mg/kg p.o., acute). No effect on general behaviour, body temperature, motor coordination and spontaneous locomotor activity and no occurrence of epileptic seizures were noticed after acute administration of S 47445 in mice (10-100 mg/kg p.o.) and rats (10-1000 mg/kg p.o.). All these results indicate that the novel AMPA positive allosteric modulator S 47445 displays cognitive-enhancing properties without CNS side-effects especially proconvulsant activity and could have promising therapeutic potential for the treatment of cognitive disorders in Alzheimer's disease.

Disclosures: **C. Louis:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **L. Danober:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **I. Carrié:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **N. Dumas:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **K. Albinet:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **K. Llopis:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **A. Roger:** A.

Employment/Salary (full or part-time):; Institut de Recherches Servier. **M. Gandon:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **A. Hugot:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **M. Krentner:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **J. Thomas:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **N. Rogez:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **M. Vandesquille:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **A. Krazem:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; contracted research. **D. Béracochéa:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research. **V. Bertaina-Anglade:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research. **C. Drieu La Rochelle:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research. **C. Junges:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **M. Bertrand:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **S. Billiald:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **C. Tordjman:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **A. Cordi:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **P. Lestage:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.07/C43

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: New insight about the mechanism of co-agonist

Authors: *L. MARGER[1], S. BERTRAND[2], D. BERTRAND[3];
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[3]HiQScreen, geneva, Switzerland

Abstract: Progresses in the field of nicotinic acetylcholine receptors were marked by a series of steps with first the identification of compounds showing enough selectivity for the different receptor subtypes followed by the discovery of their mode of action. Data obtained at the α7 nAChRs are a typical example of the recurrent interaction between basic science and translational medicine with the finding that compounds comprising a quinuclidine moiety can show a selective agonistic activity at the α7 receptors and the subsequent identification of new mechanisms of action such as the co-agonist activity (Prickaerts et al., 2011). First observed at subnanomolar concentrations of EVP-6124 (encenicline), which causes a potentiation of the response of the human α7 receptors evoked by 40 μM ACh, the co-agonist activity is thought to occur when one molecule of compound is binding to the orthosteric binding site causing a more efficacious activity triggered by exposure to a low concentration of acetylcholine. Subsequently, it was shown that co-agonist activity is not restricted to partial agonists such as EVP-6124, but that providing adequate experimental paradigm and concentrations, potentiation was also observed with nicotine and to a lesser extend with PNU 282987. To explore further these mechanisms of co-agonist activity, experiments were conducted at other nAChRs subtypes, using different experimental paradigms conducted at receptors expressed in Xenopus oocytes. Furthermore, additional experiments were designed and conducted using patch clamp recordings in whole cell configuration. Results from these two approaches are compared with reference compounds and analyzed in the light of a mathematical model. References Prickaerts J, van Goethem NP, Chesworth R, Shapiro G, Boess FG, Methfessel C, Reneerkens OA, Flood DG, Hilt D, Gawryl M, Bertrand S, Bertrand D, König G (2011) EVP-6124, a novel and selective α7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of α7 nicotinic acetylcholine receptors. Neuropharmacology 62:1099-1110.

Disclosures: L. Marger: None. **S. Bertrand:** None. **D. Bertrand:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant MH083911

NIH Grant AG045656

PSU stem cell fund

Title: Increased GABA inhibition impairs long-term potentiation and memory in a mouse model of Alzheimer's disease

Authors: *Z. WU[1,2], Z. GUO[2], G. CHEN[2];
[1]Huck Life Sci., University Park, PA; [2]Huck Inst. of Life Sci., University Park, PA

Abstract: Keywords: Alzheimer's disease, $GABA_A$ receptor, GABA inhibition, long-term potentiation, memory deficit. **Abstract** Alzheimer's disease (AD) is a debilitating neurodegenerative disorder afflicting millions of elderly people yet still with no effective therapy. Amyloid plaques and tau tangles are common pathological hallmarks for AD, however reducing Aβ production by γ-secretase inhibitors failed to relieve the symptoms of AD patients. Here, we report an enhanced GABA inhibition in a mouse model for AD (5xFAD) that results in impaired long-term potentiation and memory deficit. We have developed a novel brain homogenate puff assay, together with immunocytochemistry and GABA ELISA assay, to demonstrate that GABA concentration is higher in the hippocampal tissue in 5xFAD mice than wild type (WT) mice. We have also confirmed a high GABA content in human AD patient brains, suggesting that high GABA level may be a novel biomarker for AD and can be developed as a potential diagnostic tool. Furthermore, in accordance with above findings, electrophysiological results show abnormal GABA inhibition in 5xFAD mice. Importantly, reducing GABA inhibition in 5xFAD mice rescued the impairment of long-term potentiation (LTP) and memory deficit. Our studies suggest that reducing abnormal GABA inhibition in the hippocampus may lead to a novel therapeutic therapy for Alzheimer's disease. This project was supported by NIH and PSU stem cell fund.

Disclosures: Z. Wu: None. **Z. Guo:** None. **G. Chen:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

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Program#/Poster#: 691.09/C45

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FAPESP Proc 2004/07933-3

FAPESP Proc 2007/02536-4

CAPES

CNPq

Title: Mechanism involved in the enhanced [3H]-ACh release induced by a quaternary derivate of l-hyoscyamine in rat cortical synaptosomes

Authors: *F. M. NOGUEIRA[1], M. T. R. LIMA-LANDMAN[1], A. J. LAPA[1,2], C. SOUCCAR[1]; [1]Pharmacol., Escola Paulista de Medicina/UNIFESP, São Paulo, Brazil; [2]Lab. of Pharmacol. and Toxicology, Amazon Biotech. Ctr., Manaus, AM, Brazil

Abstract: Abnormalities in central cholinergic neurotransmission have been associated with cognitive impairments and neurodegenerative diseases like Alzheimer´s disease and Parkinson's disease. This makes the cholinergic system a potential therapeutic target for developing new compounds with specific actions. In a previous study, we have shown that a quaternary derivate of l-hyoscyamine, phenthonium (Phen), enhanced the spontaneous ACh release without affecting the nerve-evoked transmitter release at the rat neuromuscular junction. The compound is a competitive muscarinic antagonist 100 times less potent than atropine, and a noncompetitive antagonist of the muscular nicotinic ACh receptor (nAChR) (Souccar et al., Br. J. Pharm. 124:1270, 1998). In this work we analyzed the mechanisms involved in the presynaptic facilitatory effect of Phen in rat cortical synaptosomes. All experimental procedures were approved by the local Animal Investigation Ethical Committee (Protocol N° 341/09). Crude synaptosomes prepared with the cerebral cortex of male rats preloaded with [3H]-Choline (0.08 μM) were superfused with Krebs-bicarbonate buffer (0.5 mL/min) at 37°C, in the presence and absence of agonists and antagonists. The results showed that superfusion of the synaptosomes samples with Phen (10-100 μM) increased the basal [3H]-ACh release in a concentration related manner. The effect was dependent on the extracellular Ca2+ concentration indicating its exocytotic nature. The effect of a mean concentration of Phen (50 μM) was decreased by 89% in the presence of the selective muscarine antagonist oxotremorine (10 μM), but it was unaffected by nicotine (1 μM). [3H]-ACh release evoked by 15 mM K+ was not altered in the presence of Phen. Blockade of voltage-dependent Ca2+ channels with CdCl2 (0.1 mM), at a concentration that inhibited K+-evoked [3H]-ACh release, did not influence the effect of Phen. Blockade of large conductance Ca2+-activated K channels (BK) with paxilline (30 μM) did not alter the facilitatory effect of Phen on [3H]-ACh release. However, Phen-induced [3H]-ACh release was abolished after blockade of small-conductance Ca2+ activated K (SK) channels by apamin (10 nM). The results indicate that the facilitatory effect of Phen on basal [3H]-ACh release is mediated by its antimuscarinic action. The effect was related to an increase of intracellular Ca2+ mobilization and activation of SK channels. This action may be a useful mechanism to enhance the cholinergic neurotransmission in neurodegenerative disorders associated with aging.

Disclosures: **F.M. Nogueira:** None. **M.T.R. Lima-Landman:** None. **A.J. Lapa:** None. **C. Souccar:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.10/C46

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Scientific and Technological Research Council of Turkey Grant 112S135

Title: Investigation of the relationship between aromatase and seladin-1 *in vivo* and *in vitro*

Authors: *H. KARAHAN[1,2], S. LULE[3], T. KUCUKKILINC[4], A. ERCAN[4], P. KELICEN UGUR[2];
[2]Fac. of Pharm. Dept. of Pharmacol., [3]Inst. of Neurolog. Sci. and Psychiatry, [4]Fac. of Pharm. Dept. of Biochem., [1]Hacettepe Univ., Ankara, Turkey

Abstract: Selective Alzheimer's disease indicator-1 (seladin-1) is an enzyme, which is responsible of cholesterol biosynthesis, decreases in vulnerable brain regions of Alzheimer's disease. It was shown that seladin-1 protects neurons from amyloid-beta and oxidative stress related apopitotic cell death through estrogen receptors. Aromatase is another enzyme, which provides estrogen formation from androgens at the last step of estrogen biosynthesis in different tissues, including the brain. It was shown that brain aromatase has regulatory effects in the nervous system by causing estrogen formation and its level also decreases in Alzheimer's disease. Because of the neuroprotective effects of aromatase and seladin-1 via estrogen and the decrease of their brain levels in Alzheimer's disease, we hypothesized that there may be a relationship between these genes and their expressions can be affected from each other. In the aim of investigating the relationship, we inhibited aromatase or seladin-1 with their specific inhibitors both *in vivo* and *in vitro*. We treated SH-SY5Y human neuroblastoma cells with aromatase inhibitor letrozole (8 μM) or seladin-1 inhibitor U18666A (10 μg/ml) for 24 hours. Inhibitors (10^{-7} mM letrozol/10^{-6} M U18666A) were injected intracerebroventricularly for 7 days to the rats. We evaluated the aromatase and seladin-1 expression levels by Western blot experiments. Letrozole caused 1.62 fold increase ($P<0.05$) in seladin-1 level in cell culture. Aromatase protein level decreased significantly ($P<0.05$) in U18666A injected rat brains however the decrease was not significant in letrozole injected group. The decrease in aromatase level in seladin-1 inhibitor treated group made us to think that there may be an interaction

between these proteins. Seladin-1 might be increasing as a compensatory mechanism in aromatase inhibited group, where the reduced neuroprotective estrogen level is considered as a stress condition in neurons. Increasing the reduced aromatase level may be targeted in Alzheimer's disease patients, whereas the increase was estimated as a protective mechanism in neuronal damage.

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Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

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Program#/Poster#: 691.11/C47

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The 5-HT_6 antagonist Lu AE58054 potentiates the effects of acetylcholinesterase inhibition on extracellular acetylcholine levels and theta and gamma oscillations in the rat

Authors: *K. F. HERRIK[1], M. A. FORASTER[2], N. RICHARD[2], M. GARMER[2], J. F. BASTLUND[2], I. E. M. DE JONG[2], A. MØRK[2];
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Abstract: The 5-HT_6 receptor is primarily localized in the brain, in areas relevant for cognition. Combining a 5-HT_6 receptor antagonist with an acetylcholinesterase inhibitor (AChEI) represents a promising new approach for the symptomatic treatment of Alzheimer's disease. A recent phase 2 trial showed that the selective 5-HT_6 receptor antagonist Lu AE58054 improved cognitive performance in patients with moderate Alzheimer's disease on stable donepezil (AChEI) treatment. Here we investigated the effects of Lu AE58054 in combination with AChEIs on hippocampal acetylcholine (ACh) levels in awake rats and on local field potential responses to electric stimulation of the brainstem reticular formation in anesthetized rats in both hippocampus and medial prefrontal cortex (mPFC). Extracellular levels of ACh of freely-moving male Sprague Dawley (SD) rats were measured in hippocampus by microdialysis and samples were subjected to LC/MS/MS analysis. In urethane anesthetized male SD rats local field potentials were recorded with tungsten electrodes in the hippocampal fissure or in the mPFC, while brainstem (nucleus pontis oralis), electrical stimulation was applied (0.3ms square pulses, 6s, 250Hz) every 100s. Recordings were transformed using a modified continuous wavelet transform to yield the power of oscillatory activity and compared between treatments. Lu

AE58054 administered alone did not affect hippocampal ACh levels. Subcutaneous injection of donepezil at 0.5 and 1.3 mg/kg increased the hippocampal ACh levels dose-dependently. Administration of Lu AE58054 (10 mg/kg po.) 2h prior to AChEI treatment significantly enhanced donepezil-induced increases in ACh. Lower doses of Lu AE58054 (1 or 5 mg/kg) were ineffective. Intravenous injection of 2 mg/kg Lu AE58054 produced a transient increase in electrically-induced gamma power in the mPFC but did not significantly affect hippocampal oscillations. Pretreatment with 1 or 2 mg/kg Lu AE58054 potentiated the effect of donepezil (0.3 mg/kg i.v.) on gamma oscillations in the mPFC. In the hippocampus, 2 mg/kg of AE58054 potentiated the theta response to 0.3 mg/kg of donepezil and sustained the gamma response to 1 mg/kg donepezil.Lu AE58054 potentiates the effects of AChEIs on ACh levels and oscillatory activity at doses assumed to result in full receptor occupancy. Such potentiation could contribute to the procognitive effects observed in donepezil-treated Alzheimer's disease patients, although other neurotransmitters are also likely to be involved.

Disclosures: **K.F. Herrik:** A. Employment/Salary (full or part-time):; Lundbeck. **M.A. Foraster:** A. Employment/Salary (full or part-time):; Lun. **N. Richard:** A. Employment/Salary (full or part-time):; Lundbeck. **M. Garmer:** A. Employment/Salary (full or part-time):; Lundbeck. **J.F. Bastlund:** A. Employment/Salary (full or part-time):; Lundbeck. **A. Mørk:** A. Employment/Salary (full or part-time):; Lundbeck. **I.E.M. De Jong:** A. Employment/Salary (full or part-time):; Lundbeck.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.12/C48

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of β1-adrenergic signaling in Alzheimer’s disease (AD)

Authors: *M. SHAMLOO[1], L. COUTELLIER[2], P. MEMAR ARDESTANI[3];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia with a severe unmet medical need. A well-defined pathological hallmark of AD is the degeneration of the neurons in the locus coeruleus (LC), the main source of noradrenaline (NA) in the brain and the drop in NA concentration correlates with the progression and extent cognitive deficit. Therefore, we studied

the role of β1-noradrenergic signaling in cognitive function to determine whether it could be used as a potential therapeutic target for AD. Our results demonstrated that activation of β1-ADR receptors using xamoterol which is a selective partial agonist, can improve cognitive function in two mouse model of AD: Thy1-APP$^{Lond/Swe}$ and 5XFAD. Our result shows that effect of xamoterol on cognitive function is through PKA pathway by increasing nuclear pCREB in the treatment group. Our results highlight that selective activation of β1-ADR may provide a new therapeutic option for AD.

Disclosures: **M. Shamloo:** None. **L. Coutellier:** None. **P. Memar Ardestani:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.14/C50

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Novo Nordisk

Title: The GLP-1 analog liraglutide improves motor function, survival rate and reduces neurofibrillary tangle load in the transgenic hTauP301L tauopathy mouse model of Alzheimer's disease

Authors: P. BARKHOLT[1], K. FABRICIUS[1], J. JELSING[1], D. TERWEL[2], H. H. HANSEN[1], C. PYKE[3], L. B. KNUDSEN[3], *N. VRANG[1];
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Abstract: In addition to its prominent role in glycemic control, glucagon-like peptide 1 (GLP-1) exhibits neuroprotective properties. Accordingly, there is amounting experimental evidence for GLP-1 analogs, including liraglutide, to enhance synaptic plasticity, counteract cognitive deficits and ameliorate neurodegenerative features in various models of Alzheimer's disease (AD). Here we characterized the potential neuroprotective effects of long-term treatment with liraglutide in a transgenic mutant tau (hTauP301L) mouse model. This model displays pathological features of the AD brain and develops age-dependent appearance of hyperphosphorylated tau aggregation, including neurofibrillary tangle (NFT) formation, accompanied by progressive severity of motor dysfunction (limb clasping) and lethargy. Liraglutide (500 µg/kg/day, s.c., q.d., n=18) or vehicle (n=17) was administered to transgenic hTauP301L mice from the age of three months and

treatment was continued for 23 weeks in total. Vehicle-dosed wild-type FVB/N mice served as normal control. The onset and severity of hind limb clasping behavior was strikingly different in liraglutide and vehicle-dosed transgenic mice. Hence, vehicle-dosed transgenic mice displayed a significantly higher risk of clasping induction. Also, while no liraglutide-treated transgenic animals exhibited severe clasping behavior, seven vehicle-dosed transgenic mice were sacrificed due to appearance (from 19 weeks of vehicle treatment) of end-stage clasping behavior. Stereological analyses further revealed that total NFT volume was closely correlated (r^2=0.8829, $p<0.0001$) to the severity of clasping behavior. Notably, liraglutide significantly reduced hindbrain NFT load in transgenic hTauP301L mice. Hence, liraglutide improves functional motor outcome which is reflected by a lowered NFT burden in a mouse model of hindbrain-associated tauopathy. These findings suggest that liraglutide would also improve functional outcome, including cognitive function, in models involving forebrain tau pathology.

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Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

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Program#/Poster#: 691.15/C51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH

State of California Public Health Service

Title: The role of zinc in Aß-dependent synaptotoxic mechanisms in Alzheimer’s disease

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Abstract: In recent years, soluble amyloid beta oligomers (AßO) have emerged as key elements in the cascade leading to synaptic dysfunction in Alzheimer’s disease (AD). Soluble AβO are present in AD brains and transgenic models, induce neuronal death, reduce synaptic density and their levels correlate better than plaque density with cognitive impairment. Previous work from our lab has shown that synaptic accumulation of AβO is enhanced by synaptic activity and synaptic zinc released during excitatory neurotransmission increases the formation and

accumulation of AβO at synaptic sites, and that the AβO-zinc interaction accelerates oligomer formation. Other recent research has shown that sequestration of synaptic zinc by AβO disrupts synaptic function, a significant finding as synaptic zinc has been demonstrated to modulate several signaling pathways, has a high affinity for AβO and accumulates in Aβ plaques in AD brain. To further investigate the dysregulation of zinc neurotransmission we used zinc transporter ZnT3 knockout (KO) mice, which specifically lack synaptic zinc. Both ZnT3 and synaptic zinc are found throughout the brain, but are highly enriched in the mossy fiber tract of the hippocampus, a critical region for learning and memory which suffers significant neurodegeneration in AD. We used hippocampal tissue and organotypic slices to investigate markers of excitotoxicity/hyperactivity and alterations in protein phosphorylation and BNDF levels. The behavioral performance of ZnT3 KO mice was assessed to determine cognitive deficits. Our results show significant age-dependent increases in markers of excitotoxic activity, including reduced calbindin expression, increased NPY expression, and mossy fiber sprouting in the hippocampus. We also found alterations in neurotrophic signaling pathways and BDNF expression, indicating that disruption of zinc neurotransmission contributes to both synaptic dysfunction and neuronal death. Hippocampal-dependent behavioral tasks show age-dependent impairments in learning and memory in ZnT3 KO mice, which was improved with treatment with anti-seizure medication. These results indicate that perturbations in zinc neuromodulation disrupt neuronal activity and compromise neuronal viability, contributing to the neuronal hyperactivity and neurodegeneration found in AD animal models and patients. Thus, therapies directed to normalize excitatory neurotransmission and zinc modulation may prove valuable to manage hyperactivity and associated neurodegenerative changes in AD.

Disclosures: **E. Vogler:** None. **J. Busciglio:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.16/C52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P01 AG14930

Title: A précised investigation of the consequences of defects in glucose metabolism on optimal functioning of neurons in Alzheimer’s disease

Authors: *A. KUMAR[1], R. R. RATAN[2];
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Abstract: A decrease in glucose metabolism is one of the earliest changes observed in patients at risk for Alzheimer's Disease (AD), and what this change in glucose means for neurons, remains obscure. The main focus of our study was to understand the consequences of decrease in glucose metabolism on major neuronal physiologic parameters such as redox status and plasticity, which are necessary for the optimal functioning of neurons. To this end, we specifically manipulated the level of glucose uptake/utilization in-vitro in primary cortical neuronal cultures and examined its consequences on oxidative vulnerability and plasticity related gene expression. To our surprise, we found that decrease in glucose metabolism, though on one hand, resulted in significant potentiation of neuronal oxidative vulnerability, yet simultaneously, also augmented plasticity related gene expression. An extensive examination of the hexose monophosphate shunt pathway, glycolysis and o-linked glycosylation downstream of intra-neuronal glucose uptake showed that all of these pathways are modified by changes in glucose. Molecular manipulations are underway to examine those pathways most significant in creating vulnerability to oxidative stress and activating adaptive gene expression associated with plasticity. Our findings raise the possibility that decreases in glucose metabolism seen at early stage of AD may worsen the redox state of neurons but simultaneously, also open a therapeutic window by inducing a compensatory pro-adaptive transcriptional program of plasticity related genes. These exciting findings indicate the significance of molecular pathways downstream of intra-neuronal glucose as one of the key regulatory pathways of both neuronal viability as well as plasticity. Our findings, further, provide us an opportunity to screen drugs targeting these pathways, which may enable us to not only enhance neuronal viability in response to oxidative stress, but also improve the neuronal plasticity with a focus towards augmenting learning and memory, one of the key problems of AD patients.

Disclosures: A. Kumar: None. **R.R. Ratan:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

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Program#/Poster#: 691.17/C53

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG 15379

Title: Novel Syt1-PS1 interactions and their implications in Alzheimer's disease pathogenesis

Authors: *K. M. ZOLTOWSKA, A. KUZUYA, M. ARIMON, X. LI, S. SVIRSKY, O. BEREZOVSKA;
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Abstract: Amyloid β 40 (Aβ40) and more aggregation prone amyloid β 42 (Aβ42) are the two major components of amyloid plaques detected in the brains of Alzheimer's disease (AD) affected individuals. These are proteolytic products of a subsequent processing of the amyloid precursor protein by the two enzymes - β- and γ-secretases. The latter enzyme is active as a complex composed of anterior pharynx-defective 1 (APH1), nicastrin (NCT), presenilin enhancer protein 2 (PEN2) and presenilin 1 (PS1), which encompasses the active site. Continuous, default or experimentally induced neuronal activity causes an increase in Aβ production, and this is strongly related to intracellular calcium flux and synaptic vesicle exocytosis. To gain further insight into the Ca2+-dependent regulation of Aβ production, we performed a mass spectrometry screen for novel PS1 interactors and found that synaptotagmin 1 (Syt1) binds directly to PS1 in high Ca2+ conditions. Syt1 is known as a calcium sensor in neurotransmitter release and is involved in trafficking of synaptic vesicles at the active zone of the synapse. The interaction was confirmed *in vitro* and *in vivo* by co-immunoprecipitation of endogenous or overexpressed wild type or mutated Syt1 and PS1, and by Förster resonance energy transfer (FRET) experiments. Next, we explored the role of Syt1 in Aβ40 and Aβ42 production using knockdown and overexpression approaches. In addition we investigated the stability and trafficking of β- and γ-secretase components by cycloheximide pulse-chase assays, western blotting and subfractionation. Our experiments demonstrate that PS1 directly binds to Syt1 in a Ca2+-dependent manner *in vitro* and *in vivo*, and that Syt1 overexpression and knockdown result in increased and decreased levels of secreted Aβ peptides, respectively. Our results suggest that PS1-Syt1 interaction may have an implication in AD pathogenesis. Hence the role of the interaction and its potential for a novel synapse-targeting therapy for AD warrant further investigations.

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Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

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Program#/Poster#: 691.18/C54

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG037337

NIH NS-083175

ADDF 20100501

Title: Abeta oligomers are pharmacologically behaved ligands displaced by small molecule antagonists

Authors: *C. SILKY[1], N. IZZO[2], K. MOZZONI[2], C. REHAK[2], R. YURKO[2], G. RISHTON[2], G. LOOK[2], H. SAFFERSTEIN[2], S. CATALANO[2];
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Abstract: Soluble Abeta oligomers are thought to play a central role in the pathology of Alzheimer's disease. We characterized the binding of oligomers and freshly prepared monomers of synthetic Abeta 1-42 peptide (total Abeta concentration = 400nM (one hour incubation at 37oC) to DIV21 primary cultures of hippocampal and cortical neurons using immunolocalization and automated imaging. The binding of synthetic Abeta oligomers to synaptic puncta on neurons is saturable and fits a single site binding model with a Kd of 512 ± 41 nM, similar to literature values. In contrast to oligomers, equal concentrations of fresh monomers result in punctate binding that is 60% to 70% lower in intensity than that of oligomers. Monomeric Abeta binding to neuronal puncta fits a two site binding model, with a high affinity site with a similar affinity to that of oligomers (Kd = 412 ± 48 nM) and a low affinity site which is effectively non-saturable (Kd > 1 mM). ELISA and Western blots indicate that small amounts of oligomer form rapidly even in freshly prepared monomer. It is likely that the high affinity synaptic puncta binding seen with fresh monomer actually consists of oligomer that forms during the course of the experiment. The non-saturable binding at this location may result from monomer binding to negatively charged cell surface molecules and subsequent polymerization to form fibrils. Addition of novel small molecule receptor antagonist CT0109 ("Pittsburgh C") to cultures before or after addition of Abeta oligomers dose-dependently eliminates oligomer binding to synaptic puncta. These studies show that Abeta oligomers function as pharmacologically-behaved ligands with saturable, high affinity binding to synaptic puncta that can be competed for with small molecules. The novel small molecules we have found are capable of preventing and displacing binding of synthetic Abeta oligomers *in vitro*, blocking the effects of Abeta oligomers on processes underlying synaptic dysfunction. This demonstrates that there is a ligand-receptor interaction underlying the pathology of Alzheimer's disease, and that this pathological process can be stopped with small molecule therapeutics.

Disclosures: **C. Silky:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. **N. Izzo:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. **K. Mozzoni:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. **C. Rehak:** A.

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Employment/Salary (full or part-time):; Cognition Therapeutics.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.19/C55

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The expression of ProSAP/Shank proteins in development and aging in healthy and Alzheimer's disease brain

Authors: *R. CHHABRA[1], J. BOCKMANN[2], T. BOECKERS[2], A. M. GRABRUCKER[1]; [1]Dept. of Neurol., [2]Inst. For Anat. and Cell Biol., Ulm, Germany

Abstract: Synaptic homeostasis is an essential phenomenon for normal functioning of the central nervous system (CNS) and alterations in synapse formation, maturation and plasticity are tightly controlled during development and aging. It is thus not surprising that an imbalance of the establishment and maintenance of synapses is an underlying factor for many synaptopathies including Alzheimer's disease (AD), the most common cause of dementia. Various recent studies revealed that the proteins of the ProSAP/Shank family act as major scaffolding elements in the postsynaptic density (PSD) of excitatory synapses and the expression level of ProSAP2/Shank3 is able to influence synapse formation. ProSAP/Shank assembly within the PSD is Zn2+ dependent and Zn2+ might be a major factor in controlling synaptic homeostasis. Intriguingly, an imbalance in brain Zn2+ levels as well as ProSAP/Shank protein levels has been associated with a variety of neuropsychological and neurodegenerative disorders. For example, Zn2+ binding by Aβ leads to synaptic loss via dysregulation of ProSAP2/Shank3 scaffold in AD. Thus, the expression of ProSAP/Shank proteins during development and aging in a brain region- and isoform specific manner may be a major indicator of the condition of a brain under investigation.Therefore, here, we performed *in vivo* studies on mouse models investigating the expression levels of ProSAP/Shank family members during development and aging in a brain region specific manner. Moreover, since Zn2+ tightly regulates the expression of ProSAP/Shank

proteins, we analyzed the levels of zinc in the studied brain regions. To this end, we performed protein biochemistry and immunohistochemistry as well as zinc staining using wild-type mice. Next, we investigate how this expression pattern is altered in various mouse models for brain disorders such as AD. Further based on our results, we currently develop novel strategies to influence observed alterations and induce the expression levels of ProSAP/Shank proteins as seen in healthy animals. To that end, we evaluate the use of nanoparticles targeting the CNS. Taken together, this study will provide new insights into many synaptopathies such as AD and hopefully provide a basis for the evaluation and screening of substances to rescue observed pathologies.

Disclosures: R. Chhabra: None. **J. Bockmann:** None. **T. Boeckers:** None. **A.M. Grabrucker:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

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Program#/Poster#: 691.20/C56

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Taiwan NSC 101-2113-M-002-019-MY2

Title: Symmetric and frequent deposition of misfolded tau oligomers at presynaptic and postsynaptic terminals in Alzheimer’s disease

Authors: *H.-C. TAI[1], B. Y. WANG[1], A. SERRANO–POZO[2], M. P. FROSCH[3], T. SPIRES-JONES[5], B. T. HYMAN[4];
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Abstract: The accumulation of neurofibrillary tangles in Alzheimer’s disease (AD) propagates with characteristic spatiotemporal patterns following brain network connections, which implies trans-synaptic transmission of tauopathy. Tau synaptic transmission has been observed in animal models, but lacks supporting molecular evidence from human neuropathology. Here we report the identification of misfolded tau oligomers inside human neuronal synapses as potential anatomical substrates for propagating tauopathy. Tau is thought to normally be an axonal protein, and widely hypothesized to mislocalize or mistraffic in AD. By isolating intact, bipartite

synapses from cortical tissues of AD subjects, misfolded tau was detected by immunofluorescence with a distribution ratio of 15.4%:16.4%:2.9% (presynaptic-only/postsynaptic-only/both). Hyperphosphorylated tau exhibited a ratio of 23.1%:26.9%:3.8%, while total tau (any form) was 34.6%:47.1%:3.8%. Non-demented controls showed total tau distribution similar to that of AD subjects, but with much less phosphorylation and misfolding. Thus tau appears not to be mislocalized, but instead adopts misfolded, oligomeric, and phosphorylated forms within both presynaptic and postsynaptic sites. Based on these observations, we propose two models for the transmission of misfolded tau at synapses.

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Poster

691. Neuropharmacology and Neurotransmission in Dementia

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Intramural Research Program NICHD

Title: Tau-dependent Kv4.2 depletion and dendritic hyperexcitability in a mouse model of Alzheimer's disease

Authors: *A. M. HALL[1], B. T. THROESCH[2], D. A. HOFFMAN[2], E. D. ROBERSON[1]; [1]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL; [2]Mol. Neurophysiol. and Biophysics Section, Eunice Kennedy Shriver Natl. Inst. for Child Hlth. and Development, Natl. Inst. of Hlth., Bethesda, AL

Abstract: The incidence of Alzheimer's disease (AD) is increasing with the aging population and an astonishing 5.2 million Americans are affected by AD, the most common cause of dementia. Cognitive impairment worsens with declining hippocampal function. Neuronal hyperexcitability occurs early in the pathogenesis of AD and contributes to network imbalance

and the seizure activity seen in AD patients. The underlying cellular mechanisms are unknown, but emerging evidence suggests that the microtubule-associated protein tau plays a crucial role. *In vitro* studies and mouse models show that tau is required for amyloid-beta (Aβ)-induced dysfunction including neuronal hyperexcitability and cognitive impairments. Additionally, a specifically dendritic role of tau in Aβ-induced dysfunction is emerging. Therefore, we hypothesized that in the hippocampus, Aβ-induced hyperexcitability originates in the dendrites and involves tau. We used patch-clamp recordings to directly examine dendritic excitability in the CA1 region of the hippocampus. We found that dendrites, but not the soma of hippocampal neurons, were hyperexcitable in the hippocampus of mice overexpressing Aβ. This dendritic hyperexcitability was associated with selective depletion of Kv4.2, a dendritically-localized potassium channel important in the regulation of dendritic excitability, synaptic plasticity, and learning and memory. Tau reduction via genetic knockout prevented both the dendritic hyperexcitability and Kv4.2 depletion in Aβ overexpressing mice. Therefore, we conclude that Aβ-induced hyperexcitability originates in the dendrites and involves a tau-dependent change in dendritic ion channel expression. Understanding tau's role in AD pathology could reveal targeted pathways for therapeutic intervention.

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Poster

691. Neuropharmacology and Neurotransmission in Dementia

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U01 AG016976

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Title: Accrual of oligomeric tau in synapses and glial reaction are more proximate correlates to cognition than plaques and tangles

Authors: *I. BARROETA-ESPAR[1], B. GÓMEZ PÉREZ-NIEVAS[1], A. MELTZER[1], M. MARQUIE[1], F. GARCÍA-POLITE[1], K. MOULDER[2], J. C. MORRIS[2], J. E. PARISI[3], R. PETERSEN[3], M. IKONOMOVIC[4], O. LÓPEZ[4], J. VONSATTEL[5], R. MAYEAUX[5], B. T. HYMAN[1], T. GÓMEZ-ISLA[1];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Washington Univ., St. Louis, MO; [3]Mayo Clin., Rochester, MN; [4]Univ. of Pittsburgh, Pittsburgh, PA; [5]Columbia Univ., New York, NY

Abstract: Although it is widely assumed that plaques and tangles are causally related to the cognitive symptoms in Alzheimer's disease (AD), observations from several studies including our own suggest that relationships between plaques, tangles and cognition are not especially strong and do not suffice to reliably predict clinical outcome on individual basis. The possibility exists that some individuals may have an intrinsic capacity to withstand the presence of the lesions without demonstrating the typical patterns of neuronal and synaptic derangement, or may respond in a distinct manner which provides protection of cognitive function. **Objective:** To test the hypothesis that aberrant accumulation and mistargeting of soluble Abeta/tau into the synapses, rather than plaques and tangles, correlates with local loss of anatomical integrity in the EC and predicts likelihood of impaired cognition in elderly individuals at Braak stages V-VI. **Methods**: Detailed postmortem quantitative histopathological and biochemical assessments were conducted in the EC of non-demented subjects with Braak 0-II (control) (N=18), non-demented with Braak V-VI (N=11) and demented with Braak V-VI (N=20). We performed stereologically-based counts of neurons, tangles (intra and extraneuronal) and reactive glial cells (GFAP+ astrocytes and CD68+ microglia), quantified Abeta plaque burden and analyzed neurite morphology and trajectory. Levels of soluble Abeta and tau species were measured in synaptoneurosomal preparations by Western Blot and ELISA. **Results**: Despite equivalent loads of Abeta plaques and tangles in the EC, demented subjects with Braak V-VI but not non-demented with Braak V-VI, exhibited significant loss of neurons, reduction in cortical thickness, increase in number of reactive glial cells and neuritic morphological changes in the EC compared to controls. Abnormal accumulation of soluble oligomeric forms of tau in the synaptic compartment further discriminated demented from non-demented subjects at Braak V-VI. **Conclusion**: Aberrant accrual of tau oligomers in the synaptic compartment and glial reaction provide improved correlation than plaques and tangles of EC anatomical disruption with clinical outcomes in subjects with high postmortem Braak stages.

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Poster

691. Neuropharmacology and Neurotransmission in Dementia

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Forschungskommission Medizinische Fakultät, Universität Düsseldorf

Title: Amyloid-beta impairs postsynaptic function of human iPSC derived neurons

Authors: *K. NIEWEG[1], A. ANDREYEVA[2], B. STEGEN[2], G. TANRIÖVER[1], K. GOTTMANN[2];
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Abstract: In patients affected with the familial form of Alzheimer's disease, an increased production of amyloid beta is thought to trigger the early onset of the disease. Studies in transgenic mice and primary neuronal cultures have suggested that the dimeric soluble form of amyloid-beta causes synaptic dysfunction long before neuronal cell death and the deposition of amyloid plaques can be observed. The availability of human induced pluripotent stem cell derived neurons allows to study these early effects in human synapses. We took advantage of this system and treated human cortical-like neurons with 7PA2 conditioned medium, containing high levels of the synaptotoxic form of amyloid-beta. Synapse function was analyzed by patch clamp recordings and by staining of cycling vesicles with an FM-dye. Quantitative analysis of overall FM staining revealed a reduction in the FM fluorescence signal upon exposure to amyloid-beta peptides. However, FM staining and FM destaining kinetics were not affected in presynaptic vesicle clusters located on the proximal dendrites of human iPSC-derived neurons. Quantitative analysis of AMPA mEPSCs revealed a significant reduction in AMPA mEPSC amplitudes upon exposure to amyloid-beta. Taken together these results indicate that amyloid-beta peptides affect primarily postsynaptivc AMPA receptor function or expression, whereas presynaptic function appeared to be more resistant to the synaptotoxic actions of amyloid-beta. In summary, our results show that human iPSC derived neurons are an innovative tool for investigating the molecular mechanisms of synapse impairment induced by amyloid-beta and can be used as a platform for the development of new therapeutic substances.

Disclosures: K. Nieweg: None. **A. Andreyeva:** None. **B. Stegen:** None. **G. Tanriöver:** None. **K. Gottmann:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 691.24/C60

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Korea Healthcare Technology R&D Project A111230

National Research Foundation of Korea 2011-0021866

Seoul National University Bundang Hospital Research Fund 03-2010-007

Title: Dysregulation of an activity-regulated microRNA, miR-188, is associated with cognitive dysfunctions in Alzheimer's disease

Authors: *H. KIM[1], K. LEE[1], O. KWON[3], S. PARK[3], M. KIM[2], Y. LEE[3], J. KIM[3], H. KIM[2]; [1]Pharmacol., Seoul Natl. Univ., Yeongeon-Dong, Jongno-Gu, Seoul, Korea, Republic of; [2]Seoul Natl. Univ., Seoul, Korea, Republic of; [3]POSTECH, Pohang, Kyungbuk, Korea, Republic of

Abstract: MicroRNAs (miRNAs) have emerged as a part of key gene regulation. In central nervous system, miRNAs have been shown to regulate development, survival, function and plasticity. Previously, we reported that miR-188 expression is induced by synaptic activity and miR-188 controls dendritic plasticity and synaptic transmission by down-regulating neuropilin-2 (Nrp-2). Nrp-2 was known to be a negative regulator of dendrtic spine formation and synaptic transmission. In the present study, we investigated the pathophysiological significance of miR-188 in Alzheimer's disease (AD). miR-188 was found to be significantly down-regulated in the cerebral cortices (premedial gyrus) and hippocampi from AD patients. In addition, Nrp-2, one of the molecular targets for miR-188, was increased in the hippocampus from AD patients, compared to those from age-matched control subjects. We demonstrate oligomeric amyloid beta peptide1-42 (Aβ1-42) significantly diminished the expression of miR-188. The addition of miR-188 rescued the reduction in dendritic spine density induced by Aβ1-42 in rat primary hippocampal neuron cultures and in mouse primary hippocampal neuron cultures prepared from 5× FAD transgenic AD model mice. It was also shown that the reduction in the frequency of miniature excitatory post-synaptic currents (mEPSCs) induced by oligomeric Aβ1-42 was restored by the miR-188. Furthermore, the impairments in cognitive function and fEPSPs observed in 7-month-old AD transgenic model mice was ameliorated via the viral-mediated expression of miR-188 in the animals. We also show that CREB regulates miR-188 expression. Taken together, the reduction of miR-188 in the brains from AD patients may contribute to the cognitive dysfunctions observed in the disease.

Disclosures: **H. Kim:** A. Employment/Salary (full or part-time):; BrainKorea21plus. 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.; grants from Korea Healthcare Technology R&D Project (A111230), the National Research Foundation of Korea (NRF), and in part by Seoul National University Bundang Hospital Research Fund. **K. Lee:** None. **O. Kwon:** None. **S. Park:** None. **M. Kim:** None. **Y. Lee:** None. **J. Kim:** None. **H. Kim:** None.

Poster

691. Neuropharmacology and Neurotransmission in Dementia

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT Grant 1130747

Title: Enhancement of P2X2 expression and Ca2+ overload induce a mitochondrial dysfunction on neurons treated with Aβ peptide

Authors: ***J. FUENTEALBA**[1], P. GODOY[2], C. BASTIDAS[2], A. RAMIREZ[3], F. SAEZ-ORELLANA[4], J. GUZMAN[3], M. CANO-ABAD[5], A. MORENO-ORTEGA[5], L. AGUAYO[3]; [1]Dept. of Physiol., Univ. de Concepcion, Concepcion, Chile; [2]Physiol., Univ. of Concepcion, Concepcion, Chile; [3]Physiol., U de Concepcion, Concepcion, Chile; [4]Physiol., U de Concepcion, COncepcion, Chile; [5]Pharmacol., Univ. Autonoma de Madrid, Madrid, Spain

Abstract: Alzheimer's disease (AD) is a senile dementia characterized by progressive neuronal dysfunction, synaptic failure and neuronal loss. The main species associated with the toxics events are soluble oligomers of amyloid-beta peptide (Aβ), generated by amyloidogenic pathway in the abnormal processing of the amyloid precursor protein (APP). The cytosolic Ca2+ overload and mitochondrial alterations have been described as a key element in the onset of Aβ toxicity, generated through the Aβ pore formation into plasma membrane. This pore is a non-selective gate to the influx/efflux of different relevant elements like ATP, glucose and others. The P2X receptors (P2XR) are located at the neuronal membranes and form cationic channels after the activation with extracellular ATP. In our laboratory we have seen that treatment with Aβ enhance the expression of the subunit P2X2 (145 ±10%), which could be activated by the ATP that leaks through the amyloide pore, potentiating the Ca2+ overload. This hypothesis was corroborated by the use of purinergic modulators as PPADS (10 μM), inducing a recovery in the

electrophysiological synaptic functions (111% ± 18% of control). This P2X2 increment was accompanied by a re-localizacion of Fe65 protein, an intracellular protein able to bind the P2X2 receptor as well to APP, showing a re-distribution mainly near to plasma membrane, suggesting that could be a support of a higher P2X2 density in the plasma membrane. In parallel, the Ca2+ influx through the P2X2 receptor, contribute to mitochondrial collapse, showing a mitochondrial disordering, that was enhancement by Aβ peptide (0.5 μM) and prevented by the use of PPADs (10 μM). Our results suggest a tight coupling between P2X2 and Fe65 to induce a Ca2+ overload and mitochondrial failure on neurons treated with Aβ.

Disclosures: J. Fuentealba: None. **P. Godoy:** None. **C. Bastidas:** None. **A. Ramirez:** None. **F. Saez-Orellana:** None. **J. Guzman:** None. **M. Cano-Abad:** None. **A. Moreno-Ortega:** None. **L. Aguayo:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.01/C62

Topic: C.03. Parkinson’s Disease

Support: Supported by UNAM-PAPIIT IA202214-2

Supported by UNAM-PAPIIT IN215114

Title: L-DOPA treatment improve motor alterations induced by manganese exposure

Authors: *J. SANCHEZ, A. GUTIERREZ-VALDEZ, J. ORDOÑEZ-LIBRADO, V. ANAYA-MARTINEZ, E. MONTIEL-FLORES, J. ESPINOSA-VILLANUEVA, P. ALEY-MEDINA, F. HUERTA-OLIVAREZ, A. TRUJILLO-MARTINEZ, A. SANCHEZ-SORIA, M. AVILA-COSTA;
Fes-Iztacala, UNAM, Estado De Mexico, Mexico

Abstract: The present study examine the effects of divalent and trivalent Manganese (Mn2+/Mn3+) mixture inhalation on rat in order to obtain a solid animal model of Parkinson disease (PD) inducing bilateral and progressive dopaminergic cell death, correlate those alterations with motor disturbances, and determine whether L-DOPA treatment improves the behavior, to ensure that the alterations are of dopaminergic origin. Wistar rats inhaled a mixture of Mn2+/Mn3+, one hour three times a week for six months. Before Mn exposure, animals were

trained to perform motor tests and were evaluated each week after the exposure. At the ending of Mn exposure, 6 rats were orally treated with 7.5 mg/kg L-DOPA. After 6 months of Mn mixture inhalation, striatal dopamine content decreased 76%, SNc showed important reduction in the number of TH-immunopositive neurons and the rats developed motor alterations, which were reverted with L-DOPA treatment, providing substantial evidence that the motor alterations induced by the inhalation of the combination of Mn2+/Mn3+ are related to nigrostriatal dopaminergic function. Our data suggest that Mn2+/Mn3+ mixture inhalation produces similar morphological, neurochemical, and behavioral alterations to those observed in PD providing a useful experimental model for the study of this neurodegenerative disorder.

Disclosures: J. Sanchez: None. **A. Gutierrez-valdez:** None. **J. Ordoñez-librado:** None. **V. Anaya-martinez:** None. **E. Montiel-flores:** None. **J. Espinosa-villanueva:** None. **P. Aley-medina:** None. **F. Huerta-olivarez:** None. **A. Trujillo-martinez:** None. **A. Sanchez-soria:** None. **M. Avila-costa:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

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Program#/Poster#: 692.02/C63

Topic: C.03. Parkinson’s Disease

Support: UNAM-DGAPA-PAPIIT IA202214-2

UNAM-DGAPA-PAPIIT IN215114

Title: In the new model of Parkinson´s disease induced by inhalation of manganese the motor alterations are develop gradually

Authors: T. IBARRA-GUTIERREZ[1], S. SANCHEZ-SORIA[1], M. MORENO-RIVERA[1], A. GUTIERREZ-VALDEZ[1], E. MONTIEL-FLORES[1], J. RAMOS-JIMENEZ[2], V. RAMIREZ-ROSAS[3], A. SIERRA-SANCHEZ[3], M. AVILA-COSTA[1], *V. ANAYA-MARTINEZ[1]; [1]Dept Neurosci, UNAM Campus Iztacala, Mexico 54090, Mexico; [2]Academia de Biología Humana, Univ. Autónoma de la Ciudad de México, D.F. 07160, Mexico; [3]Fisiologia, Biofisica y Neurociencias, CINVESTAV-IPN, MEXICO DF, Mexico

Abstract: Recently we reported that mice and rats that were exposed to the inhalation of manganese chloride (MnCl2) and manganese acetate (MnOAc3) mixture developed movement

abnormalities, significant loss of Substantia nigra pars compacta (SNc) dopaminergic neurons, dopamine depletion and improved motor behavior in reaching task and beam walking test with L-Dopa treatment as Parkinson disease (PD) patients. To explore more about the development of the alterations induced by the inhalation of manganese in the rats (0.04 M MnCl2 and 0.02 M MnOAc3, 1 h three times a week for 6 months) we evaluated a set of motor tests at 3 and 6 months of exposure, the tests were rotarod, open field and electromyography (EMG) recordings. After 6 months, we performed on midbrain the TH immunostaining and the neurons in the SNc were counted. It is important to stand out that motor disturbances become more evident at more time of Mn inhalation (6 vs 3 months). Our results showed that Mn inhalation group decreased the time expended in the rotarod at all the revolutions per minute, reduced the distance traveled on the open field test (-62% at 6 months) and reduced the number of TH (+) neurons in the SNc by about 60%, but did not show changes in the in the EMG activity. With these data it is clear that Mn-mixture inhalation model gradually induced motor impairments similar to what happens in patients with Parkinson disease, becoming as a suitable alternative for exploring the disease.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson’s Disease

Support: USPHSG #NS074014

USPHSG #DA033121

Title: Acute administration of pramipexole increases phosphorylation of GSK-3β in the striatum of rats in an early-stage model of Parkinson’s disease

Authors: *S. TEDFORD[1], A. PERSONS[1], T. C. NAPIER[1,2];
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[2]Dept. of Psychiatry, Rush Univ., Chicago, IL

Abstract: Parkinson's disease (PD) is a motor disorder characterized by loss of dopamine in the nigrostriatal pathway and is commonly treated with dopamine D2/D3 receptor (D2/D3R) agonists (e.g., pramipexole; PPX). Dopamine D2/D3Rs are pleiotropic, and one signaling cascade includes the Akt/GSK-3β pathway wherein activation of Akt results in inhibition of GSK-3β via phosphorylation of the serine 9 residue. The Akt/GSK-3β pathway regulates many cellular processes, including cell survival and synaptic plasticity. As a result, interest in this pathway as a potential therapeutic target has increased. Expression of D2/D3Rs are altered in PD, and the effects of dopamine agonists on GSK-3β signaling in the PD brain state have not been studied. To fill this knowledge gap, adult male Sprague-Dawley rats were rendered PD-like by bilateral injections of 6-OHDA into the dorsolateral striatum. Acute administration of PPX dose-dependently improved motor deficits imposed by the 6-OHDA-induced lesions with 0.1mg/kg threshold. A separate group of rats were treated with saline or PPX (0.03, 0.3 and 0.6mg/kg), and 40min post-injection, striatal tissue was harvested. The ratio of pGSK-3β (inactive)/GSK-3β (total) was assessed by Western blot. Pilot studies conduct thus far suggest an increase in the pGSK-3β/GSK-3β ratio in rats treated with 0.6mg/kg PPX. Ongoing studies are determining the effects of PPX on other components of the Akt/GSK-3β signaling cascade. These data suggest a contribution of GSK-3β in D2/D3R-dependent signaling in the PD brain state.

Disclosures: **S. Tedford:** None. **A. Persons:** None. **T.C. Napier:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

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Program#/Poster#: 692.04/C65

Topic: C.03. Parkinson's Disease

Support: NRF (Korea) MSIP 2009-0083538

BK21+

Title: Silibinin attenuates dopaminergic neuronal loss in the Parkinson's disease mouse model by stabilizing mitochondrial membrane potential

Authors: Y. LEE, J. KIM, S. JI, H. CHUN, K. LEE, *J. LEE;
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by a selective loss of dopaminergic neurons in the nigrostriatal pathway. A lipophilic compound MPTP can cross the blood-brain barrier (BBB), and then is metabolized into the toxic MPP^+, which interferes with mitochondrial complex I of the electron transport chain, which causes cell death. Accordingly, various studies demonstrated that mitochondrial dysfunction plays a critical role in MPTP-induced PD model. This study is the first report that silibinin has neuroprotective effects on the murine MPTP model of PD. The flavonoid silibinin is the major active constituent of silymarin, an extract of the milk thistle seeds and known to have hepatoprotective, anti-cancer, anti-oxidative effects and neuroprotective effects. Administration of silibinin was effective to attenuate motor deficit and dopaminergic neuronal loss caused by MPTP. Glial activation was observed in striatum and in substantia nigra of mice treated with MPTP, but silibinin had no effects on glial activation in MPTP-induced PD mice. Furthermore, *in vitro* study confirmed that silibinin prevented MPP^+-induced reduction of cell viability and disruption on mitochondrial membrane potential in primary cultured neurons. In conclusion, the present study suggests that silibinin directly protects neurons on the MPTP-induced PD models, and this neuroprotective effect of silibinin might be mediated by stabilizing mitochondrial membrane potential. These results suggest that silibinin has mitochondrial targeting protective effects in MPTP-induced PD models and that it could be developed as a therapeutic candidate to ameliorate PD symptoms.

Disclosures: Y. Lee: None. **J. Lee:** None. **J. Kim:** None. **S. Ji:** None. **H. Chun:** None. **K. Lee:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson's Disease

Support: Ralph W. and Grace M. Showalter Research Trust Fund grant # 10098191

Title: Impairments in attentional set-shifting in a rat model of Parkinson's disease

Authors: *A. TRUONG[1], K. OTTO[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder in which dopaminergic neurons are progressively lost from the brain. The classic symptoms used to diagnose PD consist

of motor impairments such as tremor, rigidity, bradykinesia, and postural instability. However, by the time these symptoms appear, more than 50% of the dopaminergic neurons have already been depleted. If PD could be detected earlier, the progression of the disease could be slowed and the quality of life of patients with PD could be improved. It is proposed that deficits in attentional set-shifting may be used to diagnose early PD. Impairments in set-shifting have been reported to be present in PD patients in the early stages of the disease. However, whether these deficits reliably precede the occurrence of the motor symptoms of PD has not been investigated. This study examines whether set-shifting impairments can be used to diagnose early PD by utilizing the traditional 6-OHDA (6-hydroxydopamine) rat model of PD with the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) and a series of behavioral tasks measuring set-shifting and motor function. By varying the dose of 6-OHDA, early and late stages of PD are represented in this model. Current results suggest that rats lesioned with 6-OHDA show impairments in the set-shifting task while gross motor function remains intact. Future work will seek to validate these findings and perhaps initiate a new field of research in preventative approaches to PD.

Disclosures: **A. Truong:** None. **K. Otto:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

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Support: NIH Grant NS070825

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the University of Pittsburgh

Title: Dopamine efflux from residual dopamine neurons is increased after intrastriatal 6-OHDA

Authors: Z. SHU[1], A. E. RUPERT[2], S. CASTRO[2], J. D. JAUMOTTE[2], A. C. MICHAEL[1], *M. J. ZIGMOND[2];
[1]Chem., [2]Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Although the loss of striatal dopamine (DA) leads to severe motor deficits in patients with Parkinson's disease (PD), these deficits do not appear until the lesion is extensive. This aspect of the lengthy preclinical phase of PD can be modeled in animals in which a toxin, such as 6-hydroxydopamine (6-OHDA), is used to selectively damage DA neurons. Using microdialysis in 6-OHDA-lesioned rats, we have previously shown that the extracellular concentration of striatal DA remains normal until tissue DA is reduced by the toxin to about 20% of control. These results led us to hypothesize that the preservation of motor function despite substantial loss of dopaminergic neurons in the preclinical phase of the disease is due in part to an increased DA release from intact DA neurons and a decreased DA uptake. This would lead to an overall increase in the delivery of DA to striatal targets by the remaining DA neurons. However, our hypothesis has been somewhat controversial. Thus, to further test the hypothesis, we have now measured evoked DA overflow by fast scan cyclic voltammetry (FSCV). To create our model of the dopaminergic component of preclinical PD, we infused 6-OHDA (4 μg) into the striatum of male rats (~250 g). After 7-10 days, a FSCV recording electrode (5-7 μm dia.) was positioned within the striatum 1.4 mm medial and 1.3 mm dorsal to the 6-OHDA infusion site, a location outside the zone of maximal DA loss as later determined by immunohistochemical analysis of tyrosine hydroxylase. The medial forebrain bundle was then stimulated (15-60 Hz) and stimulation-evoked changes of DA levels in the extracellular space were monitored via the voltammetric electrode. At a stimulus frequency of 60 Hz, electrically evoked overflow of DA in the striatum of 6-OHDA-lesioned animals was indistinguishable from that in sham lesioned animals. This was true both before and after administration of nomifensine (20 mg/kg i.p.), an inhibitor of high affinity DA uptake. In contrast, at stimulus frequencies of 15 and 30 Hz, evoked DA overflow was substantially enhanced in the 6-OHDA animals as compared to sham-lesioned animals. This was also seen both before and after nomifensine administration, suggesting that the enhanced response to lower frequency stimulation seen in the lesioned striatum was attributable at least in part to increased DA release from residual terminals. These results support our hypothesis that partial DA lesions result in enhanced delivery efficiency of DA to synaptic targets. This may serve a compensatory function and help to explain the relative absence of motor deficits in patients with only moderate losses of DA neurons.

Disclosures: Z. Shu: None. **A.E. Rupert:** None. **S. Castro:** None. **J.D. Jaumotte:** None. **A.C. Michael:** None. **M.J. Zigmond:** A. Employment/Salary (full or part-time):; University of Pittsburgh. 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.; NINDS.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

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Topic: C.03. Parkinson's Disease

Support: the National Natural Science Foundation of China (No. 81271250)

Title: Effects of pedunculopontine nucleus stimulation on gait improvement in different 6-hydroxydopamine Parkinsonian rats

Authors: *W.-M. ZHANG[1], M. ZHOU[2], P. WEN[2], Y. YANG[2], W. ZHENG[2], J. WANG[2]; [1]Dept Neurosurg., [2]Dept. of Neurosurgery,Zhujiang hospital, Southern Med. Univ., Guangzhou, China

Abstract: BACKGROUND and OBJECT: Gait deficits are important clinical symptoms of Parkinson's disease (PD) and recent studies have shown that deep brain stimulation (DBS) of the pedunculopontine nuclear (PPN) can significantly improve PD patients with postural instability and gait disorders. However, gait deficits and the mechanism of PPN-DBS are rarely studied in clinical and basic science experiments. METHODS: We evaluated gait changes in different 6-hydroxydopamine (6-OHDA) rat models with injection of caudate putamen (CPU) or medial forebrain bundle (MFB) or substantia nigra compact (SNC) by using a computer-assisted CatWalk system. Correlations of gait parameters with tyrosine hydroxylase protein levels in the CPU and SNC were also investigated. In addition, we use the CatWalk system to access the effect of DBS in pedunculopontine tegmental nucleus (PPTg) on gait improvement of the PD rats. RESULTS: Statistical analysis showed the unilateral 6-OHDA rats had experienced significant impairment in maximum contact area, mean intensity, stride length, swing speed, stance, step cycle, duty cycle, terminal dual stance and base of support. The gait readouts in the MFB and SNC group were mostly significantly impaired while the CPU is milder and more unstable and many gait parameters showed a close correlation with the protein levels of TH. In addition, the regular step sequence and support formula changed in the the 6-OHDA+electrode group compared with the 6-OHDA group. However, under the condition of the PPTg-DBS treatment all the parameters above had no changed. During the PPTg-DBS, the stride and swing speeds were significantly increased and the stance and max contact (%) were significantly decreased in all four paws. Couplings value and the run speed variation decreased and cadence increased when the PPTg-DBS was underwent. CONCLUSION: Our study suggests that the 6-OHDA rats induced by injection in the MFB are more propitious to study gait dysfunction than the other two models. Implantation of PPTg electrodes may change the regular step sequence and support formula. The PPTg-DBS can effectively improve gait function of unilateral PD rat models and the CatWalk system can provide reliable and objective criteria to stratify gait

changes arising from 6-hydorxydopamine-induced lesion rats, which may hold promise in the study of disease progression and a new therapeutic method.

Disclosures: **W. Zhang:** A. Employment/Salary (full or part-time):; Zhujiang Hospital, Southern Medical University. **M. Zhou:** None. **P. Wen:** None. **Y. Yang:** None. **W. Zheng:** None. **J. Wang:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson’s Disease

Support: FAPESP Grant 07/50524-5

CAPES

Laboratory of Stochastic Stereology and Chemical Anatomy (LSSCA-University of São Paulo)

Title: The cardiac denervation induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) results in structural changes in myocardium of mice

Authors: ***T. H. SASAHARA**, M. MACHADO;
Animal Morphology and Physiol., UNESP, Jaboticabal, Brazil

Abstract: Toxic animal models of Parkinson’s disease (PD) are vastly used to reproduce the pathological and behavioural changes of the human disease. One of the most used neurotoxin is the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), well known as the gold standard model of PD. Studies have demonstrated cardiac sympathetic dysfunction in MPTP animal model similar to reported in PD patients. Impairments in cardiac noradrenergic nerves as well as in extra-cardiac noradrenergic innervation have been reported. In order to investigate the consequences of this dysfunction in myocardium structure, we evaluated the left ventricle of MPTP-treated mice by using 3-D design-based stereological methods. The C57/BL mice received a dose of 50mg/kg (i.p), twice, 16 h apart. This dose has been reported to induce almost the complete depletion of nigrostriatal dopaminergic cells and cardiac dysfunction, in previous studies. Animals from control group received saline solution (0.9%) (i.p), following the same application schedule as the MPTP group. Mice from both groups were euthanized seven days

after the last application. The parameters investigated were: the left ventricle volume, the total number of cardiomyocyte nuclei, the total number of cardiomyocyte, the cardiac muscle fiber total volume (CMF) and the cardiac interstitial total volume (CI). The left ventricle volume, the total number of cardiomyocyte nuclei, the total number of cardiomyocyte and the CMF total volume decreased 0.94%, 14%, 24% and 2.3%, respectively and CI total volume increased 5.4% in MPTP-treated mice group when compared to the control group. The cardiac denervation, induced by the use of MPTP in mice, resulted in left ventricular loss of cardiomyocytes without significant variation in the total number of cardiomyocyte nuclei and in the left ventricle volume, including the CMF total volume and CI total volume.

Disclosures: T.H. Sasahara: None. **M. Machado:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

Title: Comparison of striatal and nigral administration of CDNF in 6-OHDA rat model of PD

Authors: *M. H. VOUTILAINEN[1], K. ALBERT[1], J.-M. RENKO[2], A. PANHELAINEN[1], R. K. TUOMINEN[2], M. AIRAVAARA[1], M. SAARMA[1];
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Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder and current therapies do not halt the progression of the disease. Neurotrophic factors(NTF) are candidates for disease modifying therapeutics that may slow down the progression of the disease and to restore dopaminergic function. CDNF (cerebral dopamine neurotrophic factor) and MANF (mesencephalic astrocyte-derived neurotrophic factor) are endoplasmic reticulum located, but also secreted proteins with unique structure and mode of action. They constitute a novel, evolutionarily conserved NTF family expressed in vertebrates and invertebrates. Intrastriatally delivered CDNF efficiently restored midbrain dopaminergic function in rodent 6-OHDA and MPTP models of PD. Objective was to compare the neurorestorative effect of CDNF when administered either into striatum (STR) or into substantia nigra (SN), or simultaneously to both places (STR + SN) at different doses. Recombinant human CDNF or corresponding vehicle was

given as a single injection two weeks after unilateral intrastriatal injection of 6-OHDA (2x10 µg). Amphetamine-induced (2.5 mg/kg, s.c.) rotational behavior and no drug-induced cylinder test were measured every two weeks. TH-positive cells from substantia nigra pars compacta (SNpc) and striatal TH-positive fiber density were analyzed at 12 weeks post lesion. Based on the results from behavioural tests, intrastriatal CDNF (3, 10 or 20 µg) and intranigral CDNF (3 µg) were equally effective in the 6-OHDA model of PD. However, combination of intranigral CDNF (3µg) with intrastriatal CDNF (10 µg) was even more effective than the intrastriatal CDNF alone. These results indicate, for the first time, that targeting both STR and the SN with CDNF-treatment may have stronger trophic and functional effects compared to the effect after administration into either place alone.

Disclosures: M.H. Voutilainen: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); : Possible author conflict of interest is indicated. Details: M.S., R.K.T. and M.H.V. are inventors of the CDNF patent, which belongs to HermoPharma Ltd. M.S. is founder and shareholder of HermoPharma. **K. Albert:** None. **J. Renko:** None. **A. Panhelainen:** None. **R.K. Tuominen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); : Possible author conflict of interest is indicated. Details: M.S., R.K.T. and M.H.V. are inventors of the CDNF patent, which belongs to HermoPharma Ltd. M.S. is founder and shareholder of HermoPharma. **M. Airavaara:** None. **M. Saarma:** Other; : Possible author conflict of interest is indicated. Details: M.S., R.K.T. and M.H.V. are inventors of the CDNF patent, which belongs to HermoPharma Ltd. M.S. is founder and shareholder of HermoPharma.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.10/C71

Topic: C.03. Parkinson’s Disease

Title: Development of a bilateral 6-OHDA rat model for the early phase of Parkinson’s disease (PD)

Authors: *A. MICHEL[1], V. BERTAINA-ANGLADE[2], F. HUSTADT[1], E. CAYRE[2], C. DRIEU-LA-ROCHELLE[2], D. SCHELLER[1];
[1]UCB Biopharma, Braine-L'Alleud, Belgium; [2]Biotrial Pharmacol., Rennes, France

Abstract: Non-motor symptoms are increasingly recognized as important in Parkinson's Disease (PD), in particular their cognitive aspects. Unlike late-stage dementia, which is mostly related to cholinergic cortical dysfunction, symptoms related to dopaminergic dysregulation such as mild cognitive impairment and fronto-temporal executive dysfunction may appear very early in the disease process. These PD specific symptoms are characterized by a lack of mental flexibility, disordered planning, working memory and learning deficits. These symptoms remain poorly controlled by current pharmacological therapies. This study aimed to develop a rat model mimicking motor and cognitive deficits observed early in the course of Parkinson's disease. To avoid any asymmetrical and akinesia-related bias in the behavioral testing, a moderate bilateral 6-OHDA lesion of the substantia nigra was performed in Lister-Hooded male rats (3µg 6-OHDA/hemisphere). Three groups of rats - untreated controls, sham-operated or 6-OHDA lesioned - were characterized for motor deficits (i.e. general activity and rotarod) and for executive function (i.e. set-shifting test with two rules: response discrimination learning and visual cue set-shifts). The dopaminergic lesion assessment showed a significant reduction in both striatal dopamine content (between 70 and 75% reduction versus sham or control rats) and striatal Tyrosine Hydroxylase immunoreactivity. Lesioned rats displayed no motor deficits, no reduction in distance traveled and no change in rearing counts in comparison to untreated controls; however, the lesioned rats displayed a significant reduction in time spent on the rotarod compared to both control groups. Cognitive results demonstrated a weak deficit in the set-shifting task. Lesioned rats were not impaired in learning the response discrimination (first rule) in comparison to sham and control rats. During the visual cue set-shift, lesioned rats required significantly more time but not more trials than sham rats to reach the learning criterion. To conclude, this rat model with partial bilateral lesion of the dopaminergic system showed impaired motor coordination in the absence of bradykinesia and identified specific difficulties in a task assessing mental flexibility.

Disclosures: A. Michel: None. **V. Bertaina-Anglade:** None. **F. Hustadt:** None. **E. Cayre:** None. **C. Drieu-La-Rochelle:** None. **D. Scheller:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.11/C72

Topic: C.03. Parkinson's Disease

Title: Sub-chronic MPTP exposure in aged mice: Motor and non-motor phenotype, *in vivo* DAT integrity and tissue pathological Parkinson's disease markers in chronic follow-up study

Authors: *R. O. PUSSINEN[1], J. HARRIS[2], C. BUENSUCESO[2], M. CERRADA-GIMENEZ[1], A. NURMI[1], T. HUHTALA[1], U. HERZBERG[2];
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Abstract: Widely used toxin models for Parkinson's disease (PD) are available, such as the MPTP model and 6-OHDA models, which have a relevant Parkinsonian-like phenotype with associated motor symptoms and deficits of the dopaminergic system. However, rodent studies are typically performed on young adults instead of aged animals, which from the clinical point of view poses a problem. In addition, in some of the toxin-induced models and depending on the toxin doses, behavioral deficits can be transient or reversible. Furthermore, there is no clear understanding of the non-motor changes, such as gastric motility or cardiac function, both reportedly affected in PD patients and for which there is a clear unmet medical need for effective therapies. Appropriate dosing of MPTP in rodents is required to see desired disturbances of the dopaminergic system, reflected in motor and non-motor performance and endpoints. Here we attempted to refine a commonly used PD disease model by using subchronic MPTP and to evaluate the model using behavioral monitoring and gastric motility assays for non-motor symptoms. Furthermore, we used nuclear imaging to evaluate dopamine active transport system (DAT). Finally, we evaluated the striatal dopamine and metabolites as well as integrity of the dopaminergic cells and α-synuclein expression from the brain samples. Twelve month-old C57Bl/6 mice were challenged with subchronic MPTP (30 mg/kg/day i.p.) for two weeks by using Alzet minipumps. Before and after exposure with MPTP the mice were tested for their performance in beam balance (BB) test at weeks 2, 6 and 10, in rotarod (RR) on week 4, 6 and 9, and also in beam traversing (BT) test on weeks 6 and 9. Also, fine motor performance by Motorater test was evaluated on week 6 to detect changes not detectable with BB, RR and BT. In addition, integrity of the dopaminergic system was evaluated at 2 and 10 weeks post-MPTP challenge for DAT by using *in vivo* SPECT/CT. Finally, brain tissues were collected for biochemical evaluation of dopamine and its metabolites in the striatum. Also, we performed histological/immunohistochemical evaluation of tyrosine hydroxylase positive cells and α-synuclein expression in substantia nigra (SN). This validation study focused on a previously published toxin-induced mouse model of PD with novel endpoints. We present data about the motor and non-motor phenotype up to 10 weeks from the onset of MPTP challenge. In addition, we present biochemical, histopathological and nuclear imaging markers during the follow-up period in aged mice.

Disclosures: R.O. Pussinen: None. **M. Cerrada-Gimenez:** None. **A. Nurmi:** None. **T. Huhtala:** None. **J. Harris:** None. **C. Buensuceso:** None. **U. Herzberg:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: MIRECC, VA PSHCS

University of the Basque County (UFI 11/32)

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Title: Depression due to locus coeruleus (LC) neuronal loss: dose-response, pharmacology and electrophysiology

Authors: *P. SZOT[1], A. FRANKLIN[1], C. M. PALOMO[2,3], Y. WANG[1], I. VIDAURRAZAGA[2], L. UGEDO[2], M. RASKIND[1];
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Abstract: Alzheimer's disease (AD) and Parkinson's disease (PD) subjects in addition to the cognitive impairment and motor dysfunction also demonstrate depression as a common co-morbid symptom. Depression is commonly observed in the early stages of both AD and PD, even before the appearance of cognitive or motor impairment. In the general population the noradrenergic nervous system is involved in depression, and in both AD and PD there is a significant loss of noradrenergic neurons in the locus coeruleus (LC). However, depletion of CNS norepineprhine (NE) concentration observed in the DBH knockout mice or by DSP4 administration does not result in depressive-like behavior, but these methods do not reduce the number of LC neurons. Initial work in our laboratory demonstrated depressive-like behavior as observed as increased immobile time with Forced Swim Test (FST) in mice when the neurotoxin 6-hydroxydopamine (6-OHDA) was injected directly into the LC, but the depression observed with 6-OHDA is predominately observed with a moderate loss of LC neurons (less than 50% loss), not with a marked loss of LC neurons (greater than 50% loss). We further examined the depressive-like behavior produced by 6-OHDA induced LC neuronal loss by examining electrophysiological changes in the surviving LC neurons and pharmacological reversal of the 6-OHDA-induced depression. Surviving LC neurons 3 weeks after the administration of 6-OHDA (5 μg/μl) directly into the LC demonstrate increased firing frequency, more irregular firing pattern together with higher percentage of cells firing in burst. These changes in the surviving neurons in the 6-OHDA treated animals were observed with approximately a 25-30% loss of LC

neurons as determined electrophysiologically by the number of active neurons per tract and tyrosine hydroxylase immunohistochemistry. The depressive-like behavior produced by a moderate loss of LC neurons due to 6-OHDA (5 μg/μl) 3 weeks later is reversed with the prior administration (~ 6 hours before FST) of L-DOPA (3 mg/kg, sc) + benserazide (15 mg/kg, sc) or L-threo-3,4-dihydroxyphenylserine (DOPS)(500 mg/kg,sc) + benserazide (0.25 mg/kg, sc). The administration of L-DOPA or DOPS did not reverse the loss of LC neurons or the reduction of NE in the prefrontal cortex and hippocampus induced by LC neuronal loss. These results indicate that a moderate loss of LC neurons changes the firing properties of the surviving neurons and that the acute administration of L-DOPA or DOPS can normalize the depressive-like behavior in the FST but not catecholamine levels.

Disclosures: P. Szot: None. **A. Franklin:** None. **C.M. Palomo:** None. **Y. Wang:** None. **I. Vidaurrazaga:** None. **L. Ugedo:** None. **M. Raskind:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.13/D2

Topic: C.03. Parkinson’s Disease

Title: MPTP mouse model of Parkinson's Disease exhibits normal outer retinal function

Authors: *M. BEGUM[1], C. R. BISHOP[2], D. Y. TS'O[1];
[1]Neurosurg., SUNY Upstate Med. Univ., Syracuse, NY; [2]Psychology, Binghamton Univ., Binghamton, NY

Abstract: _Parkinson's disease (PD) is a neurodegenerative disorder with a wide range of motor and non-motor symptoms. Aside from the more common non-motor features like dementia, mood disturbance, hyposmia and autonomic failure, visual dysfunctions are also seen in patients with PD. These retinal dysfunctions may be a consequence of the loss of dopamine (DA) neurons in the retina, paralleling the well-known DA loss in other brain areas in PD. The overarching hypothesis of the study is that PD leads to significant abnormalities in retinal functioning including metabolism, vascularization, circulatory dynamics, that can be visualized and monitored using non-invasive retinal imaging techniques. The ability to visualize these aspects of retinal pathology in PD patients will lead to important insights as to the impact of PD on retinal function, and the origins of neuronal injury in PD. Such measurements may also possibly be used as biomarkers in clinical applications to diagnose PD and monitor PD therapy. Previous

studies have demonstrated marked functional and neuroanatomical alterations in the PD retina in human patients. It is expected that these changes have correlates that can be observed with circulatory, structural, hemodynamic and/or metabolic retinal optical imaging. _In wild-type C57BL6 mice, the technique of intrinsic signal functional imaging has demonstrated decreases in retinal reflectance that correlate with a response to visual stimuli, paralleling imaged signals also seen in other species (cat, monkey, humans). We have now use this same functional imaging method to examine the retina in the mouse MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) model of PD. _This MPTP-treated mouse model of PD recapitulates the early motor signs of PD and displays moderate but significant dopamine cell loss. This phenotype is particularly compelling given that it represents, in the mouse, an early phase of the disease state, when motor function is not severely altered and biomarkers such as abnormalities in retinal function would inform best treatment and/or neuroprotective strategies. We have imaged the retinae of these MPTP mice and a preliminary analysis has not shown any significant difference between retinal imaging signals seen in these MPTP mice and those observed in wild-type mice. These preliminary results may indicate that destruction of retinal dopamine neurons has little effect on either the outer retina or on neurovascular coupling mechanisms. In addition, preliminary retinal oximetry results suggests a lower venous oxygen saturation in the MPTP mouse retina.

Disclosures: **M. Begum:** None. **C.R. Bishop:** None. **D.Y. Ts'o:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.14/D3

Topic: C.03. Parkinson’s Disease

Title: MPTP-induced mouse model of Parkinson’s disease impairs reconsolidation and enhances extinction in contextual fear memory

Authors: *K.-I. KINOSHITA, Y. MUROI, T. ISHII;
Obihiro Univ., Hokkaido, Japan

Abstract: Parkinson’s disease (PD) is associated with progressive neurodegeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), a region of the midbrain. The most obvious symptoms are movement-related behavioral deficits including shaking, rigidity, and slowness of movement. In advanced stages of the disease, thinking and retrieval

deficits often arise from cognitive impairments. However, the mechanism of cognitive disorders in PD remains largely unknown. In the present study, we investigated the mechanism of cognitive deficits resulting from loss of the nigrostriatal dopaminergic neurons. PD model mouse (PD mouse) was produced by intraperitoneal administration of 1-methyl-4-phenyl-1, 2, 3, 6, tetrahydropyridine (four injections at a single dose of 20 mg/kg every 2 h), which destroys specifically the dopaminergic neurons in the SNpc, and the number of tyrosine hydroxylase positive cells in the SNpc was significantly decreased in PD mice compared to control (administration of saline). We evaluated the cognitive function of PD mice by the contextual fear conditioning test (CFCT). In CFCT, mice were conditioned with the footshock as unconditioned stimulus (US) in the conditioning box (context) as conditioned stimulus (CS) and 24 h later, re-exposed to CS for 3 min (reconsolidation training) or 30 min (extinction training) without US. These memories were assessed by evaluation of freezing behavior under placing mice once again in the conditioning context 24 h after re-exposure. In this study, we conducted the test using weak US (1 mA/2 s, single) or intense US (2 mA/2 s, twice). In extinction memory test under the weak US, there was no significant difference in the percentage of freezing between control and PD mice. In reconsolidation memory test, however, PD mice showed significant reduction of freezing rate compared to control. Next we carried out the experiment under the intense US. In this test, memory reconsolidation of PD mice normally occurred. However, this memory of PD mice was attenuated earlier than control by brief exposures to CS (3 min) every 24h. In extinction test PD mice also showed significant a reduction in freezing rate earlier than the control. These results suggest that MPTP-induced mouse model for PD impairs reconsolidation and enhances extinction in the CFCT, and these memory deficits may be involved in the cognitive deficits in PD patients.

Disclosures: **K. Kinoshita:** None. **Y. Muroi:** None. **T. Ishii:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.15/D4

Topic: C.03. Parkinson’s Disease

Support: Neurodyn inc

Title: The progressive BSSG rat model of Parkinson's disease: Featuring prodromal indications, early asymmetry, progressive development, anatomical spread of synuclein aggregates, and late-stage cognitive deficits

Authors: *H. A. ROBERTSON[1,2], C. A. SHAW[3], D. BARANOWSKI[2], D. G. KAY[2], J. VAN KAMPEN[2];
[1]Dalhousie Univ. Fac Med., Halifax, NS, Canada; [2]Neurodyn Inc., Charlottetown, PE, Canada; [3]Ophthalmology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The development of effective neuroprotective therapies for Parkinson's disease (PD) has been severely hindered by the notable lack of an appropriate animal model for preclinical screening. Indeed, most models currently available are either acute in nature or fail to recapitulate all characteristic features of the disease. Here, we present a novel progressive model of PD, with behavioural and cellular features that closely approximate those observed in patients. Chronic exposure to dietary phytosterol glucosides has been found to be neurotoxic. When fed to rats, *β*-sitosterol *β*-d-glucoside (BSSG) triggers the **progressive** development of parkinsonism, with clinical signs and histopathology beginning to appear *following cessation* of exposure to the neurotoxic insult and continuing to develop for several months. Here, we further characterize the progressive nature of this model, its non-motor features, the anatomical spread of synucleinopathy, and response to levodopa administration. Adult male Sprague Dawley rats received daily feedings of either plain flour pellets or flour pellets containing BSSG (3 mg) for 4 months. Animals were monitored for locomotor activity, coordination, olfaction, and cognitive function beginning immediately following cessation of toxin exposure and continuing throughout the duration of the study. Animals were sacrificed at 4, 6, 8, and 10 months following initial toxin exposure. Tissues were assayed for the loss of dopaminergic neurons, appearance of inflammatory cytokines, proteasome activity and abnormal protein aggregates. Chronic exposure to BSSG resulted in the progressive loss of nigrostriatal dopaminergic neurons. At approximately 4 months following initiation of BSSG exposure, animals displayed the early emergence of an olfactory deficit in the absence of significant dopaminergic nigral cell loss or locomotor deficits. Locomotor deficits developed gradually over time, initially appearing as locomotor asymmetry and developing into akinesia/bradykinesia; this was reversed by levodopa treatment. Late-stage cognitive impairment was observed in the form of spatial working memory deficits, as assessed by the radial arm maze. In addition to the progressive loss of TH^+ cells in the substantia nigra, the appearance of insoluble intracellular α-synuclein aggregates was also observed to develop progressively and spread from olfactory bulb to substantia nigra and, finally, hippocampal and cortical regions. The slowly progressive nature of this model, together with its *construct*, *face* and *predictive* validity, make it ideal for the screening of potential neuroprotective therapies for the treatment of PD.

Disclosures: H.A. Robertson: A. Employment/Salary (full or part-time):; Neurodyn inc. 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.; Neurodyn inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn inc. **C.A. Shaw:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn inc. **D. Baranowski:** A. Employment/Salary (full or part-time):; Neurodyn inc. **D.G. Kay:** A. Employment/Salary (full or part-time):; Neurodyn inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Inc. **J. Van Kampen:** A. Employment/Salary (full or part-time):; Neurodyn inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn inc.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.16/D5

Topic: C.03. Parkinson’s Disease

Title: Phosphatidylcholine and sphingolipid lipid alterations in the substantia nigra of 6-OHDA treated rats

Authors: *K. FARMER[1], C. A. SMITH[1], S. HAYLEY[1], J. C. SMITH[2];
[1]Neurosci., [2]Chem., Carleton Univ., Ottawa, ON, Canada

Abstract: Parkinson’s disease (PD) is a progressive neurodegenerative disease affecting the dopaminergic nigrostriatal pathway. PD patients typically do not manifest motor symptoms until 50-80% of the dopaminergic neurons have degenerated. As such, it is of great importance to determine early neuronal changes that characterize the prodromal PD phase and might contribute to disease progression. Recent attention has focused on lipids and their role in pro-apoptotic (eg. lysophosphatidylcholines) and anti-apoptotic (eg. sphingolipids) processes. However, there is a lack of information regarding the specific lipid alterations in animal models of PD. In this study, we utilized tandem high-performance liquid chromatography (HPLC) and electrospray ionization triple quadrupole mass spectrometry using a novel HPLC solvent methodology to profile phosphatidylcholines and sphingolipids within the substantia nigra of rats treated with the neurotoxin, 6-hydroxydopamine (6-OHDA). A low dose of 6-OHDA was used to provide a partial lesion, as would be expected in prodromal PD. Tissue samples were collected from the substantia nigra of Sprague Dawley rats 21 days after an infusion of either 6-OHDA (20µg) or

vehicle solution into the right anterior dorsal striatum. We subsequently identified 115 different lipid species from their mass/charge ratio using the LMAPS Lipid MS Predict Database. Interestingly, 55 lipid species (47.8%) were significantly altered in the 6-OHDA treated animals, with 58.2% of these species being up-regulated and 41.8% being down-regulated. Phosphatidylcholine lipids were evenly modified, while lysophosphatidylcholines were primarily down-regulated, and nearly all sphinganine and sphingosine species were up-regulated. These findings provide a glimpse of the lipids altered in early stages of PD-like pathology and could provide novel targets for early interventions in PD.

Disclosures: K. Farmer: None. **C.A. Smith:** None. **S. Hayley:** None. **J.C. Smith:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant R00 NS076524

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Title: A novel dopamine depletion paradigm: Comparison of behavioral deficits following bilateral gradual and acute administration of 6-hydroxydopamine in mice

Authors: *A. M. WILLARD, R. S. BOUCHARD, A. H. GITTIS;
Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Parkinson’s disease (PD) is a movement disorder whose cardinal motor symptoms arise due to loss of dopaminergic innervation of the striatum. Currently there is no well-established model that allows for incremental dopamine depletion within the same animal. This type of gradual dopamine depletion model would be useful in studies aimed at the prodromal phase of PD, when dopamine levels are pathologically low but motor symptoms have not yet presented. Utilizing the highly characterized neurotoxin 6-hydroxydopamine (6-OHDA), we have developed a paradigm in which dopamine levels in the striatum are linearly reduced over a 2-3 week time period in C57BL/6 mice. Dopamine depletion is achieved by administration of five low dose injections of 6-OHDA through an implanted intracranial bilateral cannula targeting the medial forebrain bundle. Levels of dopamine within the striatum following each dose were

quantified using tyrosine hydroxylase immunostaining and high-performance liquid chromatography. The onset and severity of motor impairments using this approach was compared with those in acutely depleted mice using the following behavioral tasks: (1) Open-field to assess general locomotion, (2) Rearing task to assess vertical activity, and (3) Pole task to test fine motor coordination. As expected, animals that had undergone gradual dopamine depletion displayed behavioral deficits when compared to saline controls across all tasks. Interestingly, these deficits were less severe than those seen in animals that had been dopamine depleted via acute high-dose injections. In open field, we observed increased freezing in dopamine depleted animals; however there was significantly more freezing observed in acutely depleted animals compared to gradually depleted animals. Similarly, in rearing, we observed decreased number of rears in dopamine-depleted animals, with acutely depleted animals rearing less than gradually depleted animals. Finally, in pole task, we observed increased latency to descend in dopamine depleted animals, with acutely depleted animals taking longer to descend than gradually depleted animals. Further study utilizing this novel dopamine depletion paradigm could uncover the time-course of plasticity that occurs at a cellular level during the prodromal phase of PD, as well as enhance our understanding of the role of dopamine in basal ganglia function.

Disclosures: **A.M. Willard:** None. **R.S. Bouchard:** None. **A.H. Gittis:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant NIGMS008306

Title: Neurodegeneration of the locus coeruleus induces hyperalgesia in sprague dawley rats

Authors: ***J. C. TOUCHETTE**, G. H. WILKEN, D. SALVEMINI, H. MACARTHUR; Pharmacol. and Physiological Sci., St. Louis Univ., Saint Louis, MO

Abstract: Parkinson’s disease (PD) is a neurodegenerative disease classified by progressive loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNpc) resulting in the inability to make controlled movements. The neuropathology of PD also affects additional brain areas, which leads to a variety of non-motor symptoms. Pain is a common complaint of up

to 70% of patients, and its causes are not well understood. The locus coeruleus (LC) of the hindbrain, the major noradrenergic nucleus of the central nervous system, is involved in descending inhibition of nociception at the level of the spinal cord. Degeneration of the LC may be the underlying cause of pain and increased sensitivity observed in PD patients. The reason(s) for the specificity of degeneration in the LC may involve the use of norepinephrine (NE) as a neurotransmitter and its oxidation into neuromelanin, similar to the environment of the SNpc. Our model of hyperalgesia involves inducing neurodegeneration of the LC by direct administration of 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPP+), a neurotoxin used for selective degeneration of catecholaminergic neurons. As LC neurons degenerate, hyperalgesia develops over time and is quantified using behavioral tests for hyperalgesia (increased sensitivity to noxious stimuli) and allodynia (pain due to a non-noxious stimulus). We found that hyperalgesia developed in these rats, while allodynia was not present. As a biochemical measurement of NE loss, we measured NE content in the cerebrospinal fluid using HPLC with electrochemical detection and found decreased levels compared to control rats. We treated a separate group of rats with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) as a positive control for selective depletion of NE exclusively from neurons originating in the LC. Future studies will determine what biomarkers of oxidative stress and/or neuroinflammation are present in the LC nucleus and in the spinal cord after treatment with MPP+. We will also determine whether therapeutic agents can protect the LC from neurodegeneration and reduce or block the development of hyperalgesia. These will include antioxidant and anti-inflammatory agents, as well as L-DOPA. Understanding the role degeneration of the LC plays in Parkinsonian pain will not only increase our knowledge of central pain in general, but may also provide insight into potential therapies for non-canonical symptoms of PD.

Disclosures: J.C. Touchette: None. **G.H. Wilken:** None. **D. Salvemini:** None. **H. Macarthur:** None.

Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.19/D8

Topic: C.03. Parkinson’s Disease

Title: Functional and morphological long-term effects of DBS in 6-OHDA hemiparkinsonian rats

Authors: *K. BADSTÜBNER[1], I. WEBER[4], A. BUSCH[2], M. WARKENTIN[2], U. GIMSA[5], D. BEHREND[2], R. BENECKE[1], J. GIMSA[3], E. MIX[1];
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Abstract: Aims: In this paper, we review long-term experiments on deep brain stimulation (DBS) in the rat 6-hydroxydopamine (6-OHDA) model of Parkinson's disease (PD). The aims of this project are to determine optimum stimulation parameters with high therapeutic efficiency, to analyze the electrode-tissue interface and to overcome the limitations of previous DBS studies in experimental rat models such as risk of disconnection of cables to external stimulators, invasive surgery for implantation of internal stimulators and/or non-representative observation periods. Methods: Unipolar platinum/iridium electrodes are inserted into the subthalamic nucleus of Wistar rats with unilateral 6-OHDA induced lesions of the medial forebrain bundle. Rats were subjected to chronic instrumentation by a portable stimulator (130 Hz, 60 µs, 200 µA) for up to six weeks. DBS effects were quantified using behavioral tests: Locomotor function was assessed by registration of the initiation time for placing forepaws in the stepping test, sensorimotor neglect by the number of retrievals in the corridor test and anxiety by recording line crossing in the open-field test. After the last test session, rats were perfused and the brains were embedded in epoxy resin to analyze the electrode-tissue interface by focused ion-beam technique. Results: Locomotor function was improved by continuous stimulation over 3 weeks and for another 3 weeks after cessation of DBS as measured by the initiation time of contralateral forepaw stepping. Surprisingly, the same effect was only seen with borderline significance by permanent DBS over 6 weeks. Similarly, the sensorimotor neglect was significantly improved by continuous DBS over 3 weeks and another 3 weeks of cessation of DBS as measured by contralateral retrievals in the corridor test whereas permanent DBS over 6 weeks showed no effect. Anxiety registered as enhanced line transition between the center and periphery of open field was reduced with borderline significance if DBS was applied for 3 weeks and for another 3 weeks after cessation of DBS whereas continuous DBS had no effect. Conclusions: Reasons for the discrepancy between interrupted and continuous DBS may be related to cellular processes at the electrode-tissue interface which will be investigated by impedance measurements and ultra-structural morphological analysis.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 692.20/D9

Topic: C.03. Parkinson's Disease

Title: 6-hydroxydopamine striatal lesions in creatine transporter deficient mice results in heightened parkinsonian symptoms

Authors: *Z. I. ABDULLA[1,3], E. R. HAUTMANN[4], K. C. UDOBI[3,2], A. N. KOKENGE[2], M. R. SKELTON[1];
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Abstract: Parkinson's disease (PD) is characterized by motor dysfunctions and resting tremors caused by the degeneration of dopaminergic neurons originating in the substantia nigra. Evidence points to the involvement of oxidative stress and mitochondrial dysfunction in the neurodegeneration observed in PD. The mitochondrial dysfunction hypothesis is supported by reduced levels of ATP in regions of the basal ganglia and midbrain of PD patients. Creatine (Cr) is a guanidine-compound produced in the kidneys, liver, and pancreas that is essential to neurological function, has important antioxidative properties, and contributes substantially to cellular energy metabolism through the creatine/phosphocreatine cycle (Cr/PCr). In addition to the reduction of ATP levels in PD patient brains, reductions of PCr - an important source of adenosine diphosphate phosphorylation - have also been observed. Cr may therefore play an essential role in the etiology of PD. In order to better understand the role of Cr in dopaminergic function related to PD, we exposed Cr Transporter -(CrT) knockout (CrT-/y), which lack Cr in the brain, and wild-type (CrT+/y) mice to unilateral striatal injections of the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA). Locomotor asymmetry and gait were assessed weekly following 6-OHDA administration. No differences in paw placement were observed in the cylinder test prior to surgery. Following 6-OHDA administration, CrT-/y mice had reduced use of the contralateral (affected) paw across all times observed. Digigait analysis indicated no differences in paw angle prior to surgery. Following 6-OHDA administration a significant increase in unaffected paw angle of CrT-/y mice was noted, potentially indicating a compensatory mechanism for impaired gait, not observed in CrT+/y mice. Taken together, the current study shows that the loss of Cr exacerbates the behavioral effects of DA depletion and highlights the important role of Cr in PD etiology.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation

Title: Impairments in gait, posture and complex movement control in rats modeling the multi-system, cholinergic-dopaminergic losses in PD

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Abstract: In addition to striatal dopamine loss, degeneration of cholinergic neurons in the basal forebrain (BF) and the brainstem pedunculopontine nucleus (PPN) were documented in patients with Parkinson's disease (PD). Loss of cholinergic projections to cortical, thalamic and midbrain regions have been associated with impairments in gait and postural control and a propensity for falls. We previously demonstrated that loss of cortical cholinergic inputs and the resulting impairments in attentional control 'unmask' gait and postural risk factors and thus yielded falls in rats with striatal dopamine loss (Kucinski et al., 2013). For this research we developed a new behavior task for the assessment of gait, postural control, and fall propensity (Michigan Complex Motor Control Task; MCMCT). Here, to determine the contributions of the PPN cholinergic projection system to complex movement control, we also lesioned the cholinergic pars compacta (posterior) division of the PPN by infusing anti-ChAT saporin-coupled immunotoxin. Rats received these lesions either in combination with BF cholinergic (192-IgG-saporin) or dorsomedial striatal dopamine loss (6-OHDA), or all three lesions together ("triples"). MCMCT performance by triples was characterized by more falls than in rats with just PPN lesions, PPN plus striatal dopamine loss, or rats with loss of both BF and PPN cholinergic neurons. High fall rates in triples persisted throughout the 20-day MCMCT testing sequence, indicating that daily practice did not improve the interactions between loss of attentional control and gait and postural deficits that underlie falls. Interestingly, combined loss of BF and PPN cholinergic neurons increased falls relative to controls and single lesions, suggesting that ascending cholinergic PPN loss sufficiently dysregulates striatal dopamine input for BF cholinergic cell loss to 'unmask' the impact of the former on striatal dysfunction. Finally, PPN cholinergic cell loss resulted in ballistic postural (recovery) movements and slip-triggered switches to asymmetrical gait. Such behavior was previously observed in rats after electrolytic lesions of the PPN region, considered a model of "Parkinsonian festination" (Cheng et al., 1981) and it may assist in maintaining balance by stabilizing the center of gravity. Collectively, our findings support the hypothesis that

PPN cholinergic projections contribute to the mediation of gait symmetry and postural control, and when lesioned in combination with forebrain cholinergic and dopaminergic system, results in profound impairments in the control of complex movements. This research was supported by the Michael J. Fox Foundation.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

Location: Halls A-C

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Program#/Poster#: 692.22/D11

Topic: C.03. Parkinson's Disease

Title: Irregularly patterned deep brain stimulation reduces pathological cortical activity in hemi-parkinsonian rats

Authors: ***S. R. SUMMERSON**, B. AAZHANG, C. T. KEMERE;
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Abstract: Deep brain stimulation (DBS) is a neuromodulation therapy currently used to treat essential tremor, dystonia and Parkinson's disease (PD), and is under investigation for treatment of many neurological disorders. Although it has been established as an effective therapy, we only have a rudimentary understanding of how to optimally design stimulation signals. The standard DBS signal for PD consists of a series of regularly spaced voltage (or current) bi-phasic square pulses of fixed amplitude and width. This design is effective, but it does not restore the neural activity to its intact state and also induces some unnatural effects, such as a narrowband power increase around the stimulation frequency due to stimulus-locked firing. We propose using a DBS signal with irregularly spaced pulses, where the pulse times are generated by adding random perturbations to the times in the regular stimulus signal. It is hypothesized that the irregularity in the pulse timing can better reduce the pathological coherence between neurons, while still maintaining the same average stimulation frequency, even over short time windows. We evaluate DBS of the subthalamic nucleus (STN) in the hemi-Parkinsonian rodent model (N = 5) using both regular and irregular stimulation patterns. Neural activity in the output layer of the primary motor cortex (M1) is recorded while stimulation is administered and the resultant changes are characterized. The changes in M1 are of particular interest because they are caused by antidromic spike propagation from STN to layer V of M1 via the hyperdirect pathway. The striatum serves as the main input nucleus to basal ganglia structures, but the hyperdirect pathway

bypasses the striatum and directly connects M1 to STN. This pathway is made up of excitatory projection neurons (pyramidal cells) from Layer V that synapse onto STN neurons. Recent work has indicated that changes in M1 activity constitute a significant part of Parkinsonian pathology and that DBS modulation of this activity may be crucial towards alleviating motor symptoms of PD. Multiple stimulation frequencies are studied so that frequency-dependent effects can be observed. We find that stimulation reduces pathological entropic noise and alters firing rate patterns in the cortex via the hyperdirect pathway to the STN. Additionally, we evaluate the LFP signal and find that there is an increase in beta band (13 - 30 Hz) power in the Parkinsonian hemisphere relative to the intact (unlesioned) hemisphere. We find that with higher frequency stimulation and irregular temporal patterns increase the corrective modulatory effect of DBS on cortical activity.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

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Program#/Poster#: 692.23/D12

Topic: C.03. Parkinson's Disease

Title: Non-motor behavioral deficits induced by a bilateral 6-OHDA lesion in the striatum of the rat

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Abstract: The progression of Parkinson's disease (PD) is typically characterized by motor impairments, as observed in standard animal models. However, this can also be accompanied by non-motor symptoms such as psychiatric disturbances, including psychosis-like symptoms. Psychosis can be facilitated by the stimulation of subcortical dopaminergic neurons or by the stimulation of serotoninergic receptors, which can induce hyperactivity and hallucinations, respectively. The present study investigated the effects of a bilateral 6-hydroxydopamine (6-OHDA) lesion in the striatum on behavior with relevance to psychosis in the rat. Dopamine or serotonin-induced behaviors were evaluated using the amphetamine-induced hyperactivity and the DOI-induced head twitch tests, respectively. Forty five rats were bilaterally injected with saline or 6-OHDA at 4 µg/µL (two infusions per striatum). Four to five weeks after the lesion, spontaneous and DOI-induced head twitches were evaluated for a period of 10 minutes. Animals

were then tested in the amphetamine-induced hyperactivity test, in which they were placed in an activity meter for a period of 30 minutes. Clozapine (5 to 8 mg/kg, s.c.) was used as a reference substance and was administered 30 minutes before testing. At the end of the experiments, the animals were perfused and the brains were collected. The lesions were confirmed by tyrosine hydroxylase (TH) fluorescent immunohistochemistry into the caudate putamen (CPu) and the substantia nigra (SN). DOI-induced, but not spontaneous head-twitches, were significantly increased in the bilateral 6-OHDA lesioned rats compared with sham-operated animals ($p < 0.01$). These symptoms were antagonized by clozapine ($p < 0.001$). In the amphetamine-induced hyperactivity test, 6-OHDA-lesioned rats showed a clear increase in activity, as measured by the number of crossings ($p < 0.001$). This hyperactivity was fully reversed by clozapine ($p < 0.001$). Brain analysis revealed a decrease of TH immunoreactivity of 40% into the CPu and of 60% into the SN in the lesioned rats as compared with sham-operated animals. These results suggest the presence of augmented DOI-induced head-twitches and hyperactivity induced by amphetamine after a bilateral 6-OHDA lesion in the rat striatum. These symptoms were antagonized by clozapine, currently the recommended treatment in PD psychosis. The present model of partial lesion of the nigro-striatal pathway may be particularly useful for evaluating non-motor symptoms associated with PD, such as dopamine and serotonin-facilitated mechanisms involved in psychosis.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

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CNPq

Title: Time-dependent behavioral effect of the Hypericum polyanthemum cyclohexane extract administration in an animal model of Parkinson´s disease induced by 6-hydroxydopamine

Authors: ***T. M. SOUZA**[1,2], M. BORSOI[2], C. BATASSINI[2], C. LAZZARETTI[2], R. B. SILVESTRIN[3], A. H. BETTI[4], C. B. ANTONIO[4], G. L. VON POSER[5], S. M. K. RATES[5,4]; [1]Dept. de Bioquímica, Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil; [2]Programa de

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Abstract: Parkinson´s Disease (PD) is a neurodegenerative disorder characterized by a loss of dopaminergic neurons in the Substantia nigra pars compacta (SNpc). New therapies for this disease are required. Since a *Hypericum polyanthemum* extract inhibited monoamine reuptake and modulated GTP binding in activated striatal dopamine receptors, the aim of this study was to evaluate, in rats, the effect of an extract from this plant in a PD model induced by 6-hydroxydopamine (6-OHDA). Wistar rats received two infusions of 6-OHDA (5.5 mL, 3 mg/mL) into the right medial forebrain bundle. A cyclohexane extract of *Hypericum polyanthemum* from aerial parts (90 mg/kg/administration) was orally administered three times spaced in 8 hours starting 4, 24, or 48 h after 6-OHDA infusion (i.e., on days 0, 1, and 2 - treatments 1, 2, and 3, respectively). Treatment 4 consisted of 2 daily administrations of the extract from day 2 to 6. Methylphenidate(MP, 20 mg/kg, i.p.)-induced ipsilateral rotations were evaluated on post-infusion days 10, 45, and 85. Tyrosine hydroxylase (TH) content in the SNpc was analyzed only in animals under treatment 2. Animals under treatment 2 and 4 rotated more ipsilaterally (384±65 and 508±81, respectively; mean±SE) than their controls (212±37 and 267±78, respectively; repeated measures ANOVA group effect, $F(1,23)=6.43$ and $p<0.02$, and $F(1,12)=5.10$ and $p<0.043$, respectively). SNpc lesion level was higher in the treated group [99.6%, 99.5-99.9% (median, IR); controls: 83%, 34-97%; Mann-Whitney test, p0.05). Therefore, when administered 3 times on the same day, the *Hypericum polyanthemum* extract had a toxic effect and altered the MP-induced rotational activity only when the treatment began 24 h after the 6-OHDA infusion. However, this increase in the rotational activity also appeared after intensifying the treatment that begun 48 h after treatment, indicating that the time window for the extract toxicity may not be closed 48 h after 6-OHDA infusion. Further data may clarify this and the underlying mechanisms that intensified the neuronal death found in the present study.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

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Program#/Poster#: 692.25/D14

Topic: C.03. Parkinson's Disease

Support: IBRO-SfN Travel Grants 2014

Title: Parkinson's disease as a consequence of traumatic brain injury: Evidence obtained in an experimental model

Authors: *P. A. DE OLIVEIRA[1], F. C. MATHEUS[1,2], J. BEN[2], M. L. SCHWARZBOLD[2], R. WALZ[2], R. D. S. PREDIGER[1];
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Abstract: The primary cause of Parkinson's disease (PD) remains unknown; although an increased number of genetic and environmental factors has been proposed, including the traumatic brain injury (TBI). In the present study, we investigated whether TBI may represent a predisposing factor to PD-related alterations in mice submitted to the systemic administration of 6-hydroxydopamine (6-OHDA), a neurotoxin unable to cross the blood-brain barrier (BBB). Swiss male mice (3 months old), were submitted to a moderate TBI on left hemisphere by free weight-drop device (12.5 g) which was confirmed 1 h later through a neurological severity score (NSS) evaluation. Then, 5 h after TBI induction, some mice were sacrificed for the analyses of BBB permeability through Evans blue overflow, while others were divided into four groups (A=control + vehicle, B=control + 6-OHDA, C=TBI + vehicle, D=TBI +6-OHDA, n= 8-10 mice per group). 6-OHDA(100 mg/kg) was injected intraperitoneally (i.p.). During a period of 4 weeks the animals were submitted to a battery of behavioral tests including the NSS, open field, rotarod, apomorphine-induced rotation and challenge with acute L-Dopa treatment. Then the animals were sacrificed for the analysis of striatal tyrosine hydroxylase (TH) expression through western blot (WB) and immunohistochemistry (IHC). All procedures were approved by local Ethical Committee in Animal Research. The moderate TBI increased ipsilateral Evans blue overflow 5 h after procedure, evidencing BBB breakdown. On the 4th week after TBI induction, significant motor and neurological impairments were observed in the animals from the TBI plus 6-OHDA group in the open field (distance travelled (m) [$F(1, 33)= 4,16$; $P\leq0,05$]), rotarod (latency to fall (s), [$F(2,66)= 5,35$, $P\leq0,05$]) and NSS (median Σ scores, group C= 2.00; D= 4.00*, * $P\leq0,05$) tests. Acute treatment with L-Dopa (25 mg/kg, i.p.) improved the motor

impairments observed in the open field (distance travelled (m), [F(1,34)= 1,67; P=0,20]) and rotarod (latency to fall (s), [F(2,66)= 2,01, P≤0,16]). No changes were observed in the apomorphine-induced rotation. Remarkably, TBI plus 6-OHDA group displayed significant reduction in striatal TH levels WB [F(1, 12)=10,42; P≤0,05] and IHC : [F(1, 22)=6,1456; P≤0,05]. Altogether, the current findings indicate that a previous moderate TBI event can disrupt the BBB, increasing the susceptibility to motor, neurological and neurochemical alterations induced by systemic administration of the dopaminergic neurotoxin 6-OHDA in mice. These results corroborate and extend previous epidemiological studies indicating that TBI may represent a relevant predisposing factor for PD.

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Poster

692. Parkinson's Disease: Animal Intoxication Models

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Topic: C.03. Parkinson’s Disease

Title: Parkinsonian motor effects induced by the VMAT-2 inhibitor tetrabenazine is exacerbated by co-administration of the SSRI fluoxetine in rodents

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Abstract: Parkinson’s disease is a neurodegenerative disorder characterized by loss of nigrostriatal dopamine (DA) neurons and depletion of neostriatal DA. Idiopathic Parkinson’s disease is a member of a family of motor disorders known as parkinsonism. The cardinal motor symptoms of parkinsonism include resting tremor, akinesia, bradykinesia, and rigidity. Drug-induced parkinsonism results from administration of agents that decrease DA transmission (e.g. DA depleting agents, DA antagonists) or increase acetylcholine levels (e.g. cholinomimetics). The motor symptoms of parkinsonism can be modeled in rodents by administration of drugs that that induce parkinsonism in humans. Tetrabenazine (TBZ) is a reversible vesicular monoamine transporter (VMAT-2) inhibitor that induces parkinsonian side effects in humans and decreases locomotion, increases catalepsy, and induces tremulous jaw movements (TJMs) when

administered to rodents. TJMs are an extensively validated rodent model of parkinsonian resting tremor and are defined as “rapid, vertical deflection of the lower jaw that resembles chewing but is not directed at any particular stimulus.” In addition to motor symptoms, PD patients also exhibit depression, anxiety, and fatigue, and are often prescribed selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (FLX; Prozac) to treat these non-motor symptoms. However, clinical reports suggest that FLX administration can exacerbate parkinsonian motor symptoms, and little is known about the neurochemical mechanisms underlying this interaction. The present study assessed the effects of co-administration of TBZ and FLX on motor symptoms, striatal DA, and DA metabolite levels in rats. Injections of TBZ induced TJMs, while co-administration of FLX with TBZ led to an additional increase in TJMs relative to TBZ alone. Locomotion was decreased by injections of FLX and the coadministration of TBZ and FLX. Additionally, DA levels in the ventrolateral striatum (VLS) were reduced after TBZ administration, and further reduced by co-administration of TBZ and FLX. DA metabolite levels in the VLS were also elevated in rats that received both TBZ and FLX. In the nucleus accumbens, TBZ administration reduced DA levels, and there was a trend towards a further reduction in DA levels after co-administration of TBZ and FLX. These results provide evidence that co-administration of TBZ and FLX decreases striatal DA levels, which could be related to the exacerbation of TBZ-induced motor dysfunctions. Furthermore, these results are consistent with clinical data reporting an increase in motor symptoms in parkinsonian patients taking FLX to treat depression.

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Poster

693. Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: NIH 1RC4NS073008-01

P50NS062684

Title: Multimodal imaging analysis of neuropathologic heterogenity in Parkinson Disease

Authors: *T. MADHYASTHA[1], J. ZHANG[2], J. LEVERENZ[4], S.-C. HU[2], T. GRABOWSKI[3]; [1]Integrated Brain Imaging Ctr., [2]Pathology, [3]Integrated Brain Imaging Center, Neurology, Radiology, Univ. of Washington, Seattle, WA; [4]Lou Ruvo Ctr. for Brain Hlth., Cleveland Clin., Cleveland, OH

Abstract: Cognitive impairments in Parkinson disease (PD) are an increasingly important limitation on quality of life. The large majority of patients with PD will eventually suffer cognitive impairment, and about 30% already are impaired at the time of diagnosis. However, profiles of cognitive impairment are heterogeneous, suggesting differential cortical involvement, and the causes and timing of cortical involvement in PD are unclear. There is growing evidence that tau and α-Synuclein (α-Syn) pathology follow a staged progression constrained by anatomical and functional connectivity. This exploratory analysis tests the hypothesis that distinct multimodal patterns of cortical thinning and perfusion visible through magnetic resonance imaging are related to underlying neuropathology (as indexed by CSF biomarkers), and differential cognitive and motor trajectories. In a pilot study, we imaged 23 subjects with early stage PD (Hoehn & Yahr stage 2), 13 of whom were classified by consensus as cognitively impaired but not demented (MCI). We identified 23 components describing patterns of increased or decreased cortical thickness and perfusion (a proxy for metabolism) using Multiple Factorial Analysis (MFA), a generalization of principal component analysis. CSF biomarkers (collected a mean of 10 months prior to imaging) were available for 13 of these subjects. No subjects showed a CSF signature of Alzheimer disease. The first component (12.9% of variance) represented temporal thinning and decreased precuneus perfusion, and was negatively correlated with Aβ42 but not cognitive impairment. A component that represented inferior parietal cortex thinning and asymmetric perfusion changes (7.9% of variance) was related to a lower ratio of tau to α-Syn, more motor symptoms, and occurrence of MCI. Finally, a small component (3% of variance) related to anterior cingulate cortex thinning and lower perfusion in the precuneus/posterior cingulate was related to higher α-Syn and executive dysfunction. These findings suggest that multimodal imaging analysis might be useful for identifying sources of heterogeneity in disease progression in PD. Notably, a pattern of cortical thinning related to Aβ42 deposition was not related to MCI in our sample, suggesting that cognitive impairment in PD is not caused solely by concurrent AD pathology. A limitation of this study is the small sample size and the PCA-based approach, which forces components to be orthogonal. Acknowledgements: NIH 1RC4NS073008-01 and P50NS062684.

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Poster

693. Parkinson's Disease: Cellular Mechanisms

Location: Halls A-C

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Program#/Poster#: 693.02/D17

Topic: C.03. Parkinson's Disease

Title: Possible role of aquaporin-4 water channels in parkinson's disease

Authors: A. PRYDZ[1], K. STAHL[1], M. PUCHADES[1], N. DAVARPANEH[1], M. NADEEM[1], V. GUNDERSEN[1], *P. J. HELM[2], M. AMIRI MOGHADDAM[1];
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Abstract: Aquaporin 4 (AQP4) is the predominant water channel in the CNS and is mainly localized in the perivascular endfeet of astrocytes, which encompass the brain microvessels. Involvement of AQP4 in formation of brain edema following stroke and hyponatremia has been shown. Furthermore, loss of the perivascular AQP4 has been associated with temporal lobe epilepsy and neurodegenerative disorders such as Alzheimer's disease. Parkinson's disease (PD) is the second most common neurodegenerative disorder. Little is known about the possible roles of AQP4 in PD. As a first step to explore the significance of AQP4 in PD, we investigated the distribution of AQP4 in mouse substantia nigra (SN) using immunofluorescence and quantitative immunogold cytochemistry. Immunofluorescence analysis showed a stronger AQP4 labeling in the non-endfeet astrocyte processes in SN compared to neocortex. Quantitative immunogold analysis supported the immunofluorescence data, showing significantly stronger immunogold AQP4 density in the perivascular and non-endfeet astrocyte processes of SN. To investigate the potential role of AQP4 in the PD pathophysiology, we studied the expression pattern of AQP4 in an acute and a sub-acute mouse-model of PD. The sub-acute mouse model was established by subcutaneous injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP), whereas intrastriatal, stereotactic, unilateral injections of the active toxic compound 1-methyl-4-phenylpyridinium (MPP+) were performed in the acute model. Injection of saline was used for the control group. One week later, the mice were perfused transcardially with a mixture of 4% paraformaldehyde and 0,1% glutaraldehyde and the brains were analyzed using confocal immunofluorescence and quantitative immunogold electron microscopy (EM). Our data show that AQP4 expression is significantly increased in the SN of both models of PD. Experiments with AQP4-knockout mice will shed more light on the potential role of AQP4 in pathpophysiology of PD.

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Poster

693. Parkinson's Disease: Cellular Mechanisms

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Title: Pathological changes in RNA binding proteins in Parkinson's disease

Authors: *C. B. TRENGROVE[1], J. KIMSZAL[1], D. IRWIN[2], T. SHUCK[2], J. TROWJANOWSKI[2], B. WOLOZIN[1];
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Abstract: The translational response to stress is mediated in part by aggregation of RNA binding proteins to form stress granules (SGs). An overactive SG pathway has been proposed as a novel therapeutic target in the field of neurodegeneration. Previous work in Alzheimer's diseased (AD) brains demonstrates co-localization of Tau pathology with the RNA binding protein (RBP) TIA-1, highlighting a significant increase in SGs in aged AD brains (Vanderweyde et. al, 2012). In the current study, we explored the response of RNA binding proteins to the pathophysiology of Parkinson's diseased (PD) and PD spectrum brains samples. We focused on the pathophysiology of neuronal changes of 9 abundant RBPs in the cingulate cortex, but are also examining other brain areas. Cortical tissue from PD brain showed striking loss of nuclear TIA-1, with a corresponding increase in cytoplasmic TIA-1. However, SGs were unexpectedly absent. Pathological correlations suggest that levels of nuclear (decreased) and cytoplasmic (increased) TIA-1 correlate strongly with coincident tau pathology, but only weakly with α-synuclein pathology. The only other RBP for which we observed disease-linked correlations, was HuD (Elav4), which is a RBP that is genetically associated with PD. We hypothesized that the low levels of SG in the PD brain, occurring despite strong TIA-1 cytoplasmic translocation, might have resulted from either a compensatory increase in degradative pathways, which might occur in PD, or interference in SG formation mediated by α-synuclein (asyn). To explore these questions, BEM17 neuroblastoma cells were transfected with fluorescently labeled TIA-1 ± asyn, and treated ± rapamycin. Activating autophagy decreased SG number and size. Interestingly, over-expressing A53T asyn also appeared to decrease SG number and size. We are currently testing the effects of exposure to exogenous oligomeric asyn. This data suggests that

the pathophysiology of PD is associated with dysfunction of the SG pathway, and could provide novel insights into mechanisms of degeneration in PD.

Disclosures: C.B. Trengrove: None. **J. Kimszal:** None. **D. Irwin:** None. **T. Shuck:** None. **J. Trowjanowski:** None. **B. Wolozin:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 693.04/D19

Topic: C.03. Parkinson’s Disease

Title: Transcriptional inhibitors Bach1 and Bach2 oppose Nrf2 function and modulate antioxidant production

Authors: *E. A. WAXMAN, B. K. JOSEPH;
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Abstract: Nuclear factor-erythroid 2 related factor 2 (Nrf2) is a transcriptional activator of the antioxidant response element (ARE), an enhancer region promoting the transcription of antioxidant enzymes such as NAD(P)H:quinine oxidoreductase 1 (NQO1), heme oxygenase 1 (HO1), and gamma-glutamyl cysteine ligase (GCL) subunits. Production of antioxidants is crucial to protect against progressive neurodegeneration and is therefore a promising mechanism for the treatment of diseases such as Alzheimer’s and Parkinson’s diseases. However, BTB and CNC homology 1 and 2 (Bach1 and Bach2) interfere with Nrf2 function, preventing the transcription of antioxidant enzymes. While Bach1 is ubiquitously expressed, Bach2 expression is limited to blood cells and neurons. We therefore tested the relative contribution of Bach1 and Bach2 in mediating antioxidant production. Using model cell lines, we examined the effects of overexpressing Bach1 or Bach2 on NQO1, HO1, GCL (both modulatory and catalytic subunits), glutathione-*S*-transferase pi, and glutathione-*S*-reductase expression. Additionally, we examined the distribution and abundance of these antioxidants in the brains of C57BL/6J mice with a genetic ablation to either Bach1 or Bach2, as compared to wild-type littermate mice. We found that Bach1 strongly regulates HO1 expression, but has limited effects on other antioxidants when Nrf2 is activated. However, Bach2, even when overexpression is limited, robustly prevents Nrf2 activity. Interestingly, genetic ablation of Bach1 or Bach2 in the brain under basal conditions has variable effects on antioxidant levels, depending on protein evaluated, cell type, and brain

region. These data suggest that Bach1 and Bach2 prevent Nrf2-mediated antioxidant enzyme expression in the brain, thereby contributing to neurotoxicity and subsequent neurodegeneration.

Disclosures: **E.A. Waxman:** A. Employment/Salary (full or part-time):; Medical Diagnostic Laboratories. **B.K. Joseph:** A. Employment/Salary (full or part-time):; Medical Diagnostic Laboratories.

Poster

693. Parkinson's Disease: Cellular Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 693.05/D20

Topic: C.03. Parkinson's Disease

Title: The sleep modulating peptide orexin-B protects midbrain dopamine neurons from degeneration

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Abstract: Orexin-A and orexin-B also known as hypocretin-1 and -2 are small hypothalamic neuropeptides derived from a common prepro-orexin precursor through proteolytic processing (Tsujino and Sakurai, Front Behav Neurosci, 2013). These peptides are involved in the control of sleep, wakefulness and energy homeostasis. The loss of orexin containing neurons in Parkinson disease (PD) is therefore likely to explain a number of sleep disturbances associated to this disorder (Fronczek et al, Brain, 2007; Thannickal et al, Brain, 2007). Here, we tested the possibility that dysfunction of the orexin system in PD may also have an impact on neurodegenerative events affecting brainstem dopamine (DA) neurons. To this aim, we used a model system of rat midbrain cultures in which DA neurons degenerate spontaneously and progressively as they mature (Toulorge et al, Faseb J, 2011). We found that orexin-B provided partial but robust protection to spontaneously dying DA neurons whereas orexin-A had only marginal effects. Rescued neurons accumulated DA efficiently by active transport suggesting that they were also functional. The effect of orexin-B was comparable in intensity to that provided by glial cell line-derived neurotrophic factor but independent of it. It was instead attributable to the activation of G protein-coupled orexin receptors and to downstream signaling events requiring intracellular calcium mobilization. In addition to its own protective action for DA neurons, orexin-B had also the potential to reveal that of the alkaloid nicotine via a

mechanism involving α7 nicotinic acetylcholine receptors. Altogether, our data suggest that a relationship may exist in PD between degenerative events affecting hypothalamic orexin neurons and brainstem DA neurons.

Disclosures: P.P. Michel: None. **S. Guerreiro:** None. **C. Florence:** None. **E. Rousseau:** None. **S. Hamadat:** None. **E.C. Hirsch:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson's Disease

Support: BBSRC/EPSRC/MRC Doctoral Training Centre in Neuroinformatics and Computational Neuroscience

Erasmus Mundus Joint Doctorate Programme EUROSPIN

HEALTH-F2-2009-241498 (EUROSPIN) to JDA / OS

Title: A systems biological approach to Parkinson's Disease

Authors: *K. F. HEIL, O. SOROKINA, J. D. ARMSTRONG;
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Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disorder with an average prevalence of approximately 0.3%, with numbers rising as populations age (1%/4% prevalence for over 60/80 year olds respectively). Initial symptoms feature disorders in motor functions followed by non-motor symptoms such as depression, amongst others. The major characteristic of the disease is the degeneration of dopaminergic neurons. Nevertheless the underlying mechanisms cannot yet be fully explained. Various molecular approaches identified key genes/proteins involved e.g. LRRK2, SNCA and PARK7. This suite of implicated genes spans multiple functional pathways, explaining the complexity of the disease. Oxidative stress, altered mitochondrial, proteasomal and lysosomal function, inflammatory changes and excitotoxicity all play a role in the disease and its pathology (Dexter, 2013). Nevertheless the whole picture in particular, the differences between causal and symptomic pathways are far from being understood. Systems biology provides a unique view of complex biological systems and

gives a framework for integrating available experimental data. Several modelling approaches have been taken with respect to PD, mainly focusing on the impaired dopamine metabolism. However, few current models, give insights into molecular mechanisms of the disease. We developed a protein-protein interaction model describing all proteins implicated in PD. The candidate protein list is based on literature and the latest human genetics studies data (hand-curated). Molecular interactions have been retrieved from public databases (Hippie, Intact, etc.). The network's community structure was obtained by applying clustering techniques (Newman, 2006). As expected, we find many PD proteins associated with presynaptic compartments. However we can see specific enrichment in subsets of proteins associated with synaptic vesicle cycling and other subsets more closely associated with post-synaptic complexes in glutamatergic neurons each of which may represent different mechanisms involved in the disease pathology.
REFERENCES: Parkinson Disease: from pathology to molecular disease mechanisms, Dexter D, et al., (2013), Free Radical Bio Med, 62, 132 Modularity and community structure in networks, Newman M, (2006), PNAS, 103, 8577

Disclosures: **K.F. Heil:** None. **O. Sorokina:** None. **J.D. Armstrong:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH Grant Z01-AG000948

Title: Regulation of the Parkinson's disease kinase LRRK2 by phosphorylation and cellular stress

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Abstract: Mutations in the gene encoding Leucine-Rich Repeat Kinase 2 (LRRK2) are a common genetic cause of Parkinson's disease (PD), but the mechanisms whereby LRRK2 is

regulated are unknown. LRRK2 is constitutively phosphorylated at Ser910 and Ser935, and phosphorylation is required for binding of 14-3-3 proteins, which in turn control the cellular localisation of LRRK2. Pharmacological inhibition of its kinase activity abolishes Ser910/Ser935 phosphorylation and 14-3-3 binding and this effect is also mimicked by several pathogenic mutations. Oxidative stress is believed to play an important role in the pathogenesis of PD while mutations in LRRK2 have been proposed to affect mitochondrial function. We hypothesised that LRRK2 is involved in the oxidative stress response and explored this by using the oxidative stressors arsenite and H2O2. Here we show that arsenite or H2O2-induced stresses promote loss of LRRK2 Ser910/Ser935 phosphorylation *in vitro*, which is reversed by phosphatase inhibition, and is observed in wild type, G2019S and kinase dead D2017A LRRK2. Arsenite-induced dephosphorylation is accompanied by loss of 14-3-3 binding and translocation of the protein to perinuclear centrosomes. Furthermore, arsenite stress promoted loss of GTP binding *in vitro* and induced LRRK2 self-association which was hindered by mutations in the ROC:COR domains. We are investigating the effects of cellular stress on LRRK2 further by utilising arsenite and other oxidative stressors to induce formation of LRRK2 complexes and decipher the differential recruitment of binding partners. Our data collectively support a role of oxidative stress in modulating LRRK2 activity, and suggest a sequence of signalling events induced by arsenite stress.

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Poster

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NIH Intramural Research Program of the National Institutes of Health, NIA (to M. R. C.)

Title: Dysregulated LRRK2 signalling cascade leading to microtubule destabilisation in Parkinson’s disease

Authors: *L. PELLEGRINI[1], D. BERWICK[2], V. SPAIN[3], J. NIXON-ABELL[3], M. COOKSON[4], K. HARVEY[3];
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Abstract: Mutations in LRRK2 are a common cause of Parkinson's disease (PD). The interaction of LRRK2 with microtubules (MTs) has pathogenic relevance, since mutations in LRRK2 segregating with PD reduce neurite outgrowth and cause an accumulation of hyperphosphorylated tau. In turn, defective post-translational modifications of tubulin and microtubule-associated proteins cause alterations in the dynamic instability of microtubules, leading to aberrant axonal transport, synaptic dysfunction and axonal degeneration. We have previously described a direct interaction between LRRK2 and β-tubulin isoforms conferred by the LRRK2 GTPase domain and modulated by familial LRRK2 GTPase domain mutants. Using molecular modelling we determined that the interaction surface is on the luminal face of microtubule (MT) protofibrils. This LRRK2-MT interaction site is in close proximity to the taxol binding and α-tubulin lysine-40 acetylation sites, both known to be important in modulating MT stability. This location is poorly accessible within mature, stabilised MTs but exposed in dynamic MT populations. Consistent with this finding, endogenous LRRK2 located to dynamic growth cones and LRRK2 knockout resulted in a significant increase in α-tubulin acetylation. New data indicate substantial cytoskeletal changes in LRRK2 knockout fibroblasts. We observed changes in cell size and form, number of filopodia, cell contacts, stress fibres, tubulin acetylation and actin content. Preliminary data also suggests changes in GSK3β expression. We are currently investigating signalling pathways important for MT stability including activation of GSK3β in knockout and mutant LRRK2 models. Taken together our data shed light on the nature of the LRRK2-MT interaction, and indicate that alterations in microtubule stability and growth cone function caused by changes in LRRK2 activity or LRRK2 mutations contribute towards the pathogenesis of PD. Investigating the signaling pathways responsible for observed changes will reveal new potential therapeutic targets.

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Poster

693. Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson's Disease

Support: Italian Ministry of Health-GR-2010-2318394

Cariplo Foundation 2012-0593

Title: Parkin regulates kainate receptor by interacting with the GluK2 subunit

Authors: *A. MARASCHI[1], A. CIAMMOLA[1], A. FOLCI[2], F. SASSONE[1], S. SATO[3], Y. OKADA[4], N. KUZUMAKI[4], H. OKANO[4], N. HATTORI[3], M. PASSAFARO[2], J. SASSONE[1]; [1]laboratory of neuroscience, Inst. Auxologico Italiano, Cusano Milanino, Italy; [2]CNR institute of neuroscience, milano, Italy; [3]Dept. of Neurol., Juntendo Univ. Sch. of Med., Tokyo, Japan; [4]Dept. of Physiol., Keio Univ. Sch. of medicine, Tokyo, Japan

Abstract: Loss of function mutations in the PARK2 gene, that encodes the protein parkin, cause autosomal recessive juvenile parkinsonism. Convincing evidence suggests that loss of parkin dysregulates synapses but the underlying molecular mechanism remains unknown. We tested the hypothesis that parkin regulates glutamate ionotropic receptors. We found that parkin interacts with the GluK2 subunit of kainate receptor (KAR). By co-immunoprecipitation and pull-down assays we identified that parkin binds to Gluk2 citoplasmic tail. Our data show that parkin modulates GluK2 levels. We found significantly increased GluK2 subunit levels in brain lysates of patients with PARK2 mutation, in brain tissue of PARK2 mouse model parkin-Q311X and in human neurons differentiated from induced pluripotent stem cell lines of PARK2 patients. We finally found that parkin can ubiquitinate GluK2. Hence, our results, suggesting that parkin modulates excitatory synaptic currents by binding to and promoting GluK2 ubiquitination, support the hypothesis that a loss of neuronal parkin increases vulnerability to excitotoxicity and suggest KAR as a new target for neuroprotective therapy in patients with the PARK2 mutation.

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Poster

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Topic: C.03. Parkinson's Disease

Support: Italian Ministry of Health-GR-2010-2318394

Cariplo Foundation 2012-0593

Title: Loss of parkin function increases KAR currents and associates with excitotoxicity

Authors: *F. SASSONE[1], A. MARASCHI[1], A. CIAMMOLA[1], A. FOLCI[2], L. MAPELLI[3], G. RONZITTI[4], L. MURRU[2], G. CAPPELLETTI[5], V. SILANI[1,6], E. CHIEREGATTI[4], M. PASSAFARO[2], J. SASSONE[1];
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Abstract: Autosomal recessive juvenile parkinsonism (ARJP) is caused by loss of function mutations in the gene PARK2 that encodes the E3 ubiquitin ligase named parkin. Previous evidence showed that parkin localized at synapses modulates synaptic protein functions and that loss of endogenous parkin makes neurons highly vulnerable to excitotoxic stimuli. This evidence leads to the hypothesis that neurodegeneration in patients with PARK2 mutations may stem from excitatory synapse vulnerability. We found that parkin silencing in *in vitro* neurons results in increased Gluk2 surface levels, KAR currents and KAR-dependent excitotoxicity. The expression of a ARJP causative parkin mutant in mouse brain results in increased KAR currents and excitotoxicity. These findings show that parkin regulates KAR function in *in vitro* e *in vivo* and suggest a role of KAR upregulation in the pathogenesis of parkin-related ARJP.

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Poster

693. Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: NRF-2012R1A1A1012435

SBRI, SMX1132521

Title: Paris(znf746) regulates levels of ribosomal rna in parkinson's disease

Authors: *H. KANG, J. LEE, J. SHIN;
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Abstract: ABSTRACT Previously, we identified that PARIS (ZNF746) transcriptionally suppresses PGC-1α in Parkinson's disease (PD) and its accumulation results in selective dopaminergic neuronal death. However, the functional knowledge on PARIS is still limited. Herein, our study indicates that PARIS interacts with 160-kDa Myb-hbinding protein 1α (p160MBP) that is account for ribosomal DNA (rDNA) transcription and ribosomal RNA (rRNA) editing. Indeed PARIS co-localizes with the components of RNA polymerase I and overexpression of PARIS suppresses rDNA transcription in dopaminergic SH-SY5Y cells. Accordingly, ChIP assay shows that PARIS occupies on the promoter of human rDNA. Furthermore tandem-affinity purification assay shows that PARIS is involved in ribosomal 40S and 60S complex suggesting its putative function on protein synthesis in cytoplasm. Therefore, we suggest that PARIS may play an important role in the rRNA metabolism, including ribosomal DNA transcription in PD pathogenesis.

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Poster

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Topic: C.03. Parkinson's Disease

Support: Colciencias 1101521128595

DIB-UNal

Title: Downregulation of Pink1 and Parkin influences the activation of tyrosine kinase receptors in dopaminergic cells

Authors: *G. ARBOLEDA, A. NIÑO, H. ARBOLEDA;
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Abstract: Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disorder PD is characterized by loss of dopaminergic neurons in the sustantia nigra pars compacta (SN) and the formation of Lewy bodies (LB). Mutations in Parkin (PARK2) and Pink1 (PARK6) genes have been identified in early-onset autosomal recessive familial forms of the disease. Pink1 and Parkin encode proteins associated to mitochondrial dynamics such as fission, fusion and mitophagy, but their exact function remains unknown. Aim: to evaluate the effect of downregulation of Pink1 and Parkin on survival pathways and neuronal metabolism in a model of dopaminergic neurons (cells CAD). Materials and methods: by using three cell lines: shControl (control line), shPink1 (with reduced Pink1 expression) and shParkin (with reduced Parkin expression) we assessed cell viability by WST and LDH; western blotting was performed against AKTp, AKTt, ERK1/2p, ERK1/2t, GSK3βp, IGF-1Rp, IGF-1Rt, IRp, IRt and hexokinase; location of hexokinase was assessed by confocal microscopy. Results: Our results show that decreased expression of Parkin and Pink1 caused loss of cell viability due to a deficiency in the phosphorylation of AKT (serine 473) and ERK1/2 (tyrosines 202/204) and its downstream targets such as GSK3 and hexokinase (HK). HK expression was decreased in cells shPink1 and increased in shParkin cells. However, both cell lines showed deficiency in the activity of hexokinase and mitochondrial localization. We also found changes in mitochondrial distribution in shPink1 and Parkin cells which were mainly localize din the perinuclear area as well as a loss of mitochondrial membrane potential. This phenotype can be explained by changes found in tyrosine kinase receptors. Conclusions: These analyses suggest that decreasing Pink1 affects phosphorylation of IGF-1 receptor (IGF-1R) while decreasing Parkin is associated with defects on the phosphorylation and activation of insulin receptor (IR. These observations opens new alternative pathways by which Pink1 and Parkin may regulate neuronal viability.Acknowledgements: Pink1 constructs were a kind gift from Dr. Mark Cookson (NIH, USA).

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Poster

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Support: SRI SBT Award W32MTD

SRI IR&D Award W32S6X

Beatrice & Roy Backus Foundation P21769

Title: miRNA and autophagy in the pharmacological Gaucher mouse model

Authors: *S. K. MAK[1], N. KO[1], V. P. CHOU[1], D. A. DI MONTE[2], A. B. MANNING-BOG[1]; [1]SRI Intl., Menlo Park, CA; [2]German Ctr. for Neurodegenerative Dis., Bonn, Germany

Abstract: This study investigates early stage pathogenetic targets that contribute to degeneration in Gaucher disease, Parkinson's disease (PD) and Gaucher-related PD. Clinical, pathological and experimental evidence provides a link between α-synucleinopathies and lysosomal storage diseases, in particular PD and Gaucher disease. Genetic screens show that GBA mutation carriers are at risk for developing parkinsonism and other α-synucleinopathies, and α-synuclein pathology (i.e. Lewy bodies) are found in post-mortem studies of such cases. Autophagy is a crucial pathway to digest the unwanted proteins to maintain protein homeostasis in the brain. However, if lysosomal clearance were impaired, toxic proteins such as α-synuclein accumulate into dopaminergic neurons and promote neurodegeneration. Here, we utilized a paradigm of glucocerebrosidase deficiency, exposing mice to 100 mg/kg conduritol B epoxide (CBE) or vehicle, i.p., once daily for 21 consecutive days. Preliminary data showed significant activation of chaperone-mediated autophagy (CMA) and macroautophagy and a markedly altered profile of miRNAs in the pharmacological mouse model. Specifically, in CBE-treated mice, an apparent increase in cellular α-synuclein protein in the nigrostriatal pathway with no change in ventral midbrain Snca transcript was detected. These changes were accompanied by significantly increased Lamp2A transcript (~1.6 fold)-- indicative of CMA activation--and macroautophagic markers such as Atg3 (~1.8 fold) and Atg12 (~1.4 fold) transcripts. On-going studies will elucidate the impact of miRNA modulation on autophagic dysfunction and α-synuclein pathology related to glucocerebrosidase deficiency. These studies were funded by SRI SBT Award W32MTD (AMB), SRI IR&D Award W32S6X (SKM) and Beatrice & Roy Backus Foundation P21769 (AMB/SKM).

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Poster

693. Parkinson's Disease: Cellular Mechanisms

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Alfried Krupp prize

Title: Compensatory T-type calcium channel activity alters dopamine D2-autoreceptor responses of dopamine Substantia nigra neurons from Cav1.3 L-type calcium channel deficient mice

Authors: *J. DUDA[1], C. POETSCHKE[1], E. DRAGICEVIC[1], J. BENKERT[1], T. P. SNUTCH[2], J. STRIESSNIG[3], B. LISS[1];
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Abstract: The progressive loss of dopamine (DA) midbrain neurons, particularly within the Substantia nigra (SN), is the pathophysiological hallmark of Parkinson's disease (PD), causing its major motor related symptoms. The cause for this selective and progressive degeneration of SN DA neurons in PD remains still unclear. However, calcium load, metabolic stress and mitochondrial dysfunction, as well as altered ion channel activity have been identified as trigger-factors for PD. Insights from mouse models indicate a causal role of Cav1.3 L-type Ca2+ channels (LTCCs) for SN DA degeneration in PD. Indeed, blood-brain-barrier permissive LTCC blockers (dihydropyridines) protect SN DA neurons from degeneration in a dose-dependent fashion in PD mouse models, and also have PD-protective effects in humans. Mechanistically, LTCCs in SN DA neurons have been linked to oscillating Ca2+ levels during pacemaker activity, creating a high intracellular Ca2+ burden, associated with mitochondrial dysfunction, oxidative stress and neurodegeneration. Although identified as key players for the complex degenerative process of PD, the exact molecular composition and the physiological roles of LTCCs in SN DA neurons remain unclear. Thus, by cell-specific RT-qPCR approaches, we quantified the mRNA-levels of both pore-forming Cav1.2 and Cav1.3 LTCCs α1-subunits that are expressed in SN DA neurons, in mouse (postnatal juvenile and adult WT and Cav1.3 KO) and human (PD and control) SN DA neurons. Complementary functional *in vitro* brain-slice patch clamp characterization of SN DA neurons from Cav1.3 KO mice suggests a compensatory phenotype in Cav1.3 KO SN DA neurons, which might explain why SN DA neurons in Cav1.3 KO mice are not protected from degeneration in a 6-OHDA PD-model (see Bock et al, Abstr. SFN 2014). Adult-like, non-desensitizing D2-AR responses were present in Cav1.3 KO mice in both, juvenile (PN13) and adult (PN90) SN DA neurons. This indicates that the constitutive

absence of Cav1.3 activity throughout development in Cav1.3 KO mice prevents desensitizing dopamine D2-autoreceptor (D2-AR) responses, present in juvenile WT SN DA neurons. The altered, non-desensitizing SN DA D2-AR response of juvenile Cav1.3 KO mice were reversed to WT-like, desensitizing D2-AR response by the T-type Ca2+ channel (TTCC) blocker Z941 (10μM) as well as by a peptide (10μM), preventing interactions of D2-AR with the neuronal calcium sensor NCS-1. These findings point to a compensatory TTCC activity in juvenile SN DA neurons from Cav1.3 KO mice, modulating their Ca2+ and NCS-1 dependent D2-AR responses. We currently quantify TTCC α-subunits (Cav3.1-3.3) in mouse SN DA neurons.

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Poster

693. Parkinson's Disease: Cellular Mechanisms

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Title: Prolyl oligopeptidase directly interacts with alpha-synuclein and enhances its dimerization

Authors: *M. H. SAVOLAINEN[1], X. YAN[2], T. T. MYÖHÄNEN[1], H. J. HUTTUNEN[2]; [1]Div. of Pharmacol. and Pharmacotherapy, [2]Neurosci. Ctr., Univ. of Helsinki, Helsinki, Finland

Abstract: A-synuclein (aSyn)-rich inclusions are the pathological feature of Parkinson’s disease. A serine protease, prolyl oligopeptidase (PREP), has been linked to aggregation and accumulation of aSyn, and pharmacological inhibition of PREP has been shown to inhibit this process. It has been proposed that PREP might serve as a nucleation point for aSyn aggregation,

although the exact mechanism remains unknown. Furthermore, we have recently shown that a PREP inhibitor, KYP-2047, increases the activity of macroautophagy pathway which is an important mechanism for cellular turnover of aSyn. Suggesting that there may be a dual mechanism for PREP in the regulation of cellular aSyn levels. Here, we hypothesized that there is a direct interaction between aSyn and PREP, and that this interaction modulates the oligomerization process of aSyn. We have used two biochemical methods to investigate protein-protein interaction between PREP and aSyn. First, we studied the interaction between PREP and aSyn with microscale thermophoresis (MST, Nanotemper Monolith NT.115), a novel cell-free method that allows determination of binding affinity of pure proteins. Secondly, we used protein-fragment complementation assay (PCA), in which complementary fragments of a luciferase reporter protein were fused with wildtype PREP, enzymatically inactive PREP(S554A) mutant and aSyn, which were then co-expressed in N2A cells. Bioluminescence, which occurs only if the proteins of interest interact, was monitored in living cells. PCA was also used to study the effects of PREP overexpression and its pharmacological inhibition with KYP-2047 on aSyn dimerization. In both MST and PCA assays, wildtype PREP and PREP(S554A) interacted with aSyn. The binding affinity (Kd) of PREP-aSyn interaction was 1.8 μM as determined by MST. In PCA, addition of KYP-2047 did not have an effect on the PREP-aSyn interaction. When aSyn dimerization was studied with PCA, we observed significant increase in bioluminescence when PREP or PREP(S554A) were co-expressed with aSyn reporter proteins indicating increased dimerization of aSyn. Addition of KYP-2047 to wildtype PREP but not PREP(S554A) expressing cells restored aSyn dimerization signal back to control level. Our data suggests that there is a direct protein-protein interaction between PREP and aSyn, and this interaction seems to be independent of enzymatic activity of PREP. Differences in gating properties of wildtype PREP and PREP(S554A) may explain why KYP-2047 only abolishes wildtype PREP-induced aSyn accumulation. Our results further support previous findings suggesting that PREP has also other cellular functions in addition to its peptidase activity.

Disclosures: **M.H. Savolainen:** None. **T.T. Myöhänen:** None. **X. Yan:** None. **H.J. Huttunen:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

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Glaucoma Foundation grant

Title: α- and γ-syn immunopathology in the brain of patients with neurodegenerative diseases

Authors: *I. SURGUCHEVA[1,2], K. NEWELL[3], A. SURGUCHOV[1,2];
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Abstract: The accumulation of misfolded proteins as inclusion bodies or aggregates is a common pathologic finding in the central nervous system in neurodegenerative disorders. The protein family consists of α- , β-, and γ-synuclein (γ-syn). α-Synuclein (α-syn) filaments form aggregates in Lewy body disorders (LBD), including Parkinson's disease and dementia with Lewy bodies (DLB), and in glia in multiple system atrophy. Formation of synuclein aggregates is facilitated by post-translational modifications, e.g. phosphorylation of α-syn and oxidation of γ-syn. Among the synucleins, only α-syn has been shown to form aggregates in LBD; however, our current studies suggest that γ-syn aggregates are also present in DLB. In DLB, intracytoplasmic Lewy body inclusions are detected in neurons in multiple brain areas, including substantia nigra. These inclusions are immunoreactive with antibodies to post-translationally modified synucleins, i.e. α-syn phosphorylated on serine 129 (phospho-α-syn), as well as with oxidized met^{38} γ-syn (oxi-γ-syn). Double staining with both antibodies reveals several different morphologically distinctive aggregates, i.e. dot-like structures and doughnut-like inclusions with round to elongated shapes. Separate immunofluorescent images obtained with individual antibodies specific to phospho-α-syn and oxi-γ-syn highlight colocalization, yet different distributions of these synuclein isoforms in substantia nigra inclusion bodies in 6 cases of DLB we have studied. Phospho-α-syn is present almost exclusively at the periphery of inclusions, whereas γ-syn immunoreactivity is also located more centrally, forming dot-like structures. Asterisk-like structures vary from 1 to 15 in a single high power field, and show immunopositivity for oxi-γ-syn. Asterisk-like structures increase with subject age. Oxidized γ-syn colocalizes with phosphorylated α-syn in doughnut-like inclusions, but does not colocalize with asterisk-like structures. These structures are not stained by antibodies to α-syn, β-amyloid, Iba1, or GFAP, or with Thioflavin S. In some cases, aggregates show colocalization with the astrocyte marker ALDH1L1. Frequencies of these structures in aged-match controls are approximately 10 times lower. Our findings suggest that accumulation of native and oxidized γ-syn may be associated with pathological structures that show a different distribution pattern and differ morphologically from those detected with α-syn. Oxi-γ-syn may be an informative new biomarker in combination with other markers, in studying protein aggregates in DLB and other LBD.

Disclosures: I. Surgucheva: None. **K. Newell:** None. **A. Surguchov:** None.

Poster

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Topic: C.03. Parkinson's Disease

Support: Branfman Family Foundation

NIH P41 GM103412

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Title: Trafficking of alpha-synuclein in neurons studied with timeSTAMP genetic probes and correlated LM and 3D EM

Authors: *D. BOASSA[1], S. PHAN[1], P. NGUYEN[1], J. HU[1], M. ELLISMAN[1,2];
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Abstract: Alterations in the human alpha-synuclein (AS) gene is linked to autosomal-dominant Parkinson's Disease (PD). In humans, either missense mutation or allele multiplication results in early onset autosomal dominant PD, also implicating AS over-expression in disease pathogenesis. Previously we used genetic probe-based labeling methods (4Cys and MiniSOG), and advanced 3D EM methods to investigate the effects of human wild-type AS overexpression both *in vitro* and *in vivo* to recapitulate the clinical circumstance of increased expression of AS associated with the pathogenesis of sporadic as well as familial PD (Boassa et al., 2013). To elucidate the subcellular distribution and trafficking of age-specific subpopulations of AS proteins with high spatial and temporal resolution, we applied a new modification to our molecular probe strategy which introduces a time-tracking capability with pulse-chase approach, TimeSTAMP-YFP-MiniSOG (Butko et al., 2012). This tool provides the ability to select samples during a live imaging study using light microscopy as labeled copies of a target protein are being positioned in their cellular subdomains, and to visualize them both by fluorescence and at high resolution by EM. By applying the time-STAMP-specific protease inhibitor (BILN-2061), we were able to visualize a pulse of newly synthesized AS proteins in time-lapse LM imaging (with short incubation time) or longer-lived AS proteins destined for degradation (with longer incubation with the inhibitor). Our findings showed that the "young" AS proteins are localized within the cell body and extend in the neurites. EM analysis, including 3D electron tomography of the labeled proteins, revealed a diffuse cytosolic localization of AS, as well as an association with intracellular membrane systems and microtubules. Our observations are consistent with previous studies showing that the transport of AS by slow component-b of axonal

transport depends on microtubules. In contrast, “older” AS proteins are mainly localized in pre-synaptic terminals associated with various membrane systems (plasma membrane, membranes of synaptic vesicles or multivesicular bodies). Due to the high sensitivity and spatial resolution of the method these studies reveal the time course of AS protein movement from the soma to the nerve terminal and back to the lysosomal system, including dynamics at a sub-synaptic level.

Disclosures: D. Boassa: None. **S. Phan:** None. **P. Nguyen:** None. **J. Hu:** None. **M. Ellisman:** None.

Poster

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS060872-05

Title: Post-transcriptional regulation of α-synuclein by LRRK2 through interactions with microRNAs in neurodegenerative diseases

Authors: *J. BOON[1], A. ABELIOVICH[2], B. WOLOZIN[3];
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Abstract: Recent studies identified alternatively spliced α-synuclein transcripts, and indicated that an isoform with an extended 3’ untranslated region exhibits increased expression in Parkinson’s disease. Studies also suggest that the α-synuclein transcript with the long 3’UTR is more toxic. LRRK2 is also thought to regulate translation. For example, interaction of LRRK2 with 4E-BP leads to an increase in translation. The 3’UTR is a target region for RNA binding proteins and microRNAs, and mediates regulation of translation. The ability of LRRK2 to regulate translation and microRNA biology led us to hypothesize that LRRK2 might regulate the expression and toxicity of α-synuclein through a mechanism involving microRNAs. Consistent with this hypothesis, we observed that LRRK2 increased α-synuclein expression when 3’-UTR (long or short; Syn-L or Syn-S) was included in the cassette. WT LRRK2 increased protein expression of GFP-tagged α-synuclein constructs containing 3’UTR (relative to constructs lacking 3’ UTR). Studies in primary neurons also showed similar results. Co-transfecting LRRK2 with GFP::α-synuclein containing long or short 3’UTR also increased expression. In the

presence of LRRK2, GFP::Syn-S yielded greater expression than GFP::Syn-L. Consistent with the higher expression, neurons expressing LRRK2 + GFP::Syn-S exhibited a greater number of α-synuclein containing puncta along the processes. These data suggest differential effects of LRRK2 on α-synuclein depending on the type of 3'UTR. Next we investigated whether regulation of α-synuclein expression by LRRK2 required the binding of microRNAs. The 3'UTR sequence of Syn-S has a binding site for miR7, while that of Syn-L has binding sites for both miR7 and miR153. To investigate the role of these miRs, we performed site-directed mutagenesis, mutating the binding sites for either miR7 and/or miR153, where they no longer can bind. Deletion of the miR7 site abolished all effects of LRRK2 on expression of Syn-S or Syn-L. In contrast, deleting the miR153 site resulted in only a moderate decrement in Syn-L expression. These results suggest that LRRK2 can modulate α-synuclein expression via sites regulated by miRs, and in particular, miR7 as the strongest mediator of LRRK2 actions. Future studies will investigate whether anti-miRs exhibit similar effects. These data implicate a novel mechanism through which LRRK2 can regulate α-synuclein biology.

Disclosures: J. Boon: None. **A. Abeliovich:** None. **B. Wolozin:** None.

Poster

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Title: MitoNEET is activated by oxidative stress resulting in kinase signaling

Authors: L. LIN, W. J. GELDENHUYS, J. G. ROSS, *R. T. CARROLL;
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Abstract: mitoNEET (CISD1) is located on the outer mitochondrial membrane that has been implicated in helping to regulate the oxidative capacity of the cell. Although mitoNEET belongs to an ancient family of zinc finger proteins, its cellular function is largely unknown. During mitoNEET immunoprecipitation experiments, we found that ERK co-purified with this protein.

During oxidative stress, we found that mitoNEET release from the mitochondria correlated with the activation of ERK (pERK) in a time dependent manner using N2α cells. In addition, we found that hydrogen peroxide (H_2O_2) induces mitoNEET expression in neuronal N2α cells while rotenone caused a decrease in mitoNEET expression. These data suggest that mitoNEET expression is regulated through oxidative insult and mitochondrial integrity. Taken together, we show an interaction among mitoNEET, ERK, and oxidative insult implying a role for mitoNEET in the cellular response against oxidative stress through kinase activation.

Disclosures: L. Lin: None. **R.T. Carroll:** None. **W.J. Geldenhuys:** None. **J.G. Ross:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

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Program#/Poster#: 693.20/D35

Topic: C.03. Parkinson's Disease

Title: Study of mitochondrial death pathways in different cellular models of Parkinson's disease: effect of beta-Estradiol

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder in the United States. 6-hydroxydopamine (6-OHDA), a H2O2 pro-oxidant a natural dopaminergic catabolite that accumulates in Parkinson's disease-affected brains that may strongly contribute to PD. 1-methyl-4-phenylpyridinium (MPP+), a mitochondrial complex I inhibitor, have been widely used to produce toxin models of sporadic PD. Chronic exposure to rotenone, a nature-derived pesticide, shows slowly progressive DA neuronal loss, and Lewy body-like particles (primarily aggregations of alpha-synuclein). These 3 toxins are used to mimic *in vitro* PD. Despite the fact of all the 3 toxins are able to block the respiratory chain complexes (mainly complex 1); they seem to have well distinct pathways leading to dopaminergic (TH) neuronal death. A deep knowledge of the cytopathologic effects of each toxin is absolutely fundamental in the process of drug discovery of neuroprotective compounds. In this study, we first carefully dissected the cytopathological toxicity of the 3 compounds and secondly the effect of 17 beta-Estradiol (B-Estr) on rat primary mesencephalic neurons injured by MPP+, 6-OHDA and Rotenone exposure was assessed. Briefly rat TH neurons were obtained from 15-day old rat embryos. The midbrains were dissociated and seeded at a density in 96 well-plates. On day 6 of

culture, the medium was removed and fresh medium was added, without or with 6OHDA, MPP+ or Rotenone diluted in control medium in presence or absence of B-Estr. We showed that each toxins despite a similar target on the respiratory chain complex, activated distinctive death pathways depending of the dose used and the time of incubation. We demonstrated that Rotenone at low doses activated apoptotic pathway, whereas at higher doses necrosis was recorded. The bioenergetics pathways were highly perturbed (dose and time dependent) in the hours following its application (ATP pool decrease). By contrast, MPP+ induced large necrosis (time and dose dependent) at the doses used for cell intoxication; apoptosis was only observed for the massive and long intoxication. No ATP pool depletion was observed. At opposite, 6OHDA showed large apoptosis and a massive oxidative stress without affecting ATP pools. Interestingly, B-Estr was able to protect TH neurons from rotenone injuries (for low doses and short time of incubation, for high doses protection was marginal) with reduction of caspase 3 activation but without affecting ATP pool depletion. Similarly, B-Estr significantly protected from MPP+ injuries by contrast was unable (whatever the doses) to protect TH neurons from 6OHDA injuries and the induced massive oxidative stress.

Disclosures: **M. Combes:** None. **N. Callizot:** None. **P. Poindron:** None.

Poster

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Topic: C.03. Parkinson’s Disease

Support: Mylan School of Pharmacy

Title: Selective vulnerability in the telencephalic cortex; implications for topographic neurodegeneration

Authors: ***J. POSIMO**, A. GLEIXNER, R. LEAK;
Duquesne Univ., Pittsburgh, PA

Abstract: The phylogenetically modern neocortex is less vulnerable to tau and alpha-synuclein inclusions than the primitive allocortex. One hypothesis to explain this topography is that protein misfolding stress (aka proteotoxicity) travels through circuits in a staggered fashion and that pathology is transmitted to the allocortex before the neocortex. An alternative hypothesis is that some regions are inherently more vulnerable than others. In order to determine whether cortical

subregions differ in their ability to withstand proteotoxicity, we applied proteasome inhibitors to primary neuronal cultures from neocortex and cultures from 3 allocortical subregions: entorhinal cortex, piriform cortex, and hippocampus. Neocortex was less vulnerable to proteasome inhibitors than cultures from any allocortical subregion. The proteasome inhibitor MG132 also raised ubiquitinated proteins and decreased proteasome activity in allocortex more than neocortex. However, allocortex was less vulnerable to paraquat, suggesting that it is more resilient to oxidative stress. One of the major defenses against proteotoxicity is the heat shock protein family. We therefore contrasted heat shock protein expression in neocortex versus allocortex *in vitro* after MG132 treatment and *in vivo* as a function of age. Allocortical cultures were much more responsive to MG132 in heat shock protein induction, as might be expected from neurons that are more severely stressed. For example, Hsp70 and Hsp32 (heme oxygenase 1 or HO1) levels were higher in allocortex upon MG132 treatment. Inhibition of Hsp70/heat shock cognate 70 (Hsc70) activity with VER155008 and HO1 activity with tin protoporphyrin (SnPPx) exacerbated the toxicity of MG132 in allocortical cultures more than neocortical cultures. These findings suggest that allocortex raises Hsp70 and HO1 to higher levels because it needs to rely on these defenses more. Unlike the *in vitro* MG132 data, age-related stress increased the expression of Hsp70 equivalently in sensorimotor neocortex and entorhinal allocortex. CHIP levels were higher in allocortex in young animals. However, CHIP and Hsp90 levels were lower in allocortex in the oldest animals. Although these findings support the hypothesis of selective vulnerability, they also suggest that allocortex has impressive defenses against oxidative and proteotoxic stress, especially when its cells are young. However, these defenses are not sufficient to render it as resilient as neocortex to proteotoxicity. Furthermore, allocortex is more vulnerable to proteotoxic stress in the absence of heat shock protein defenses and its chaperone defenses may collapse in old age.

Disclosures: J. Posimo: None. **A. Gleixner:** None. **R. Leak:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: National Institute of Neurological Disorders and Stroke intramural program

Title: The role of Parkin on defective mitochondria in *Drosophila*

Authors: *C.-H. HUANG, R. J. YOULE;
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Abstract: Mitochondrial DNA (mtDNA) point mutations and deletions accumulate during aging in tissues and are linked to the pathogenesis of common diseases, such as cancer, diabetes, and neurodegenerative disorders. Recently, our group found that Parkin, which is commonly mutated in recessive familial forms of Parkinson's disease (PD), promotes the degradation of dysfunctional mitochondria (mitophagy) *in vitro*. Our previous work in cell lines with both wild-type and mutant mtDNA has shown that increasing Parkin levels influences the ratio of deleterious mtDNA mutations. However, the influence of Parkin on the frequency and pathological effects of mtDNA mutations is unknown in an intact metazoan model system. To address this question, we created a fly model that has a higher mtDNA mutation rate to determine the effects of Parkin. We successfully generated a mutator fly model by expressing a proofreading-deficient form of polymerase gamma (Polg) in a Polg null or heterozygous background. Mutator flies have elevated mtDNA point mutation levels, decreased lifespan and a locomotion defect. Parkin loss-of-function mutations induced lethality in the mutator background. Conversely, defects in lifespan and locomotion in the mutator fly are significantly suppressed by increasing the Parkin gene dosage. Our findings support the model that Parkin functions in mitochondrial quality control *in vivo* and provides a causative link between mtDNA mutations and PD pathogenesis.

Disclosures: C. Huang: None. **R.J. Youle:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson's Disease

Title: Using LUHMES cells as a model system to study dopaminergic neuron cell biology and Parkinson 's disease

Authors: *T. H. DAVIS[1], A. BAL[2], J. FOULKE[2], L. CHEN[2], F. TIAN[2];
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Abstract: Dopaminergic neurons play significant roles in motor, reward and motivational behavior related circuits throughout the brain. To date, there are few, continuous *in vitro* models

available to laboratories in research industry and academia for studies related to basic dopaminergic cell biology or high throughput screening. Here, we propose the use of a human model system, LUHMES cells, to study dopaminergic neuron cell biology and Parkinson's disease. LUHMES cells are neuronal precursors derived from human fetal ventral mesencephalon. Neuronal differentiation is governed by the termination of v-myc expression using low levels of tetracycline. During our characterization, we found that tetracycline induced differentiation resulted in robust neurite outgrowth in LUHMES cells within two to four hours. One day post differentiation, cells displayed similar morphology, with several long processes protruding from the cell soma. Growth cones were often observed in early differentiated cultures. Immunocytochemistry in early differentiated cultures (DIV 2-3) revealed low level expression of tyrosine hydroxylase, however these levels were increased significantly by 7 DIV with many neurons expressing tyrosine hydroxylase. We also investigated dopamine transporter expression. Differentiated LUHMES cultures were positive for neuronal markers such as β-tubulin and devoid of expression of traditional glial markers including GFAP and IBA-1. Both undifferentiated and differentiated LUHMES cells were easily transfected using basic eGFP constructs, although greater efficiencies were observed with the use of viral constructs. In summary, LUHMES cells are an inexpensive, yet robust *in vitro* model system that should be considered when studying dopaminergic neuron cell biology and mechanisms underlying Parkinson's disease.

Disclosures: **T.H. Davis:** A. Employment/Salary (full or part-time):; ATCC. **A. Bal:** A. Employment/Salary (full or part-time):; ATCC. **J. Foulke:** A. Employment/Salary (full or part-time):; ATCC. **L. Chen:** A. Employment/Salary (full or part-time):; ATCC. **F. Tian:** A. Employment/Salary (full or part-time):; ATCC.

Poster

693. Parkinson's Disease: Cellular Mechanisms

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Field Neurosciences Institute

John G. Kulhavi professorship in Neuroscience

Title: The co-culturing of dopamine-like cells with glia to improve cellular replacement treatment in Parkinson's disease patients

Authors: *J. W. WATTERS[1,2], R. WELCHKO[1,2], G. SHALL[1,2], S. PARKER[1,2], M. JEAKLE[1,2], L. SIEGAL[1,2,3], J. ROSSIGNOL[1,2,4], M. LU[1,2,3], G. DUNBAR[1,2,3,5];
[1]Field Neurosciences Inst. Lab. For Resto, Mount Pleasant, MI; [2]Program in Neurosci., Mount Pleasant, MI; [3]Dept. of Psychology, Central Michigan Univ., Mount Pleasant, MI; [4]Col. of Med., Mount Pleasant, MI; [5]Field Neurosciences Inst., Saginaw, MI

Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder that currently has no cure. It is characterized by the progressive loss of dopaminergic (DA) neurons, especially in the substantia nigra, causing significant motor impairments. Current therapies can alleviate some of the symptoms, but do not adequately treat the underlying problem. However, cell replacement therapy, using stem cells, offers great promise in treating PD patients by restoring the lost DA cells. In the brain, DA cells are continuously provided with the protein glial-derived neurotrophic factor (GDNF), non-neuronal glial cells. GDNF has been shown to improve survivability and growth of DA neurons. Because of this benefit, most current protocols involving using stem cells to create DA neurons include adding recombinant GDNF to the media, which does not fully replicate the function that glia serve. In addition, protocols that utilize recombinant GDNF are costly. Some studies have indicated that a more appealing option is to co-culture astrocytes with the DA cells, prior to their transplantation, in order to more naturally supply the DA cells with GDNF. This *in vitro* study compared two techniques of producing high quantities of DA cells for the use in transplantation. The number of surviving cells in culture using a co-culture technique was compared to the established method of adding trophic supplement (GDNF). The survivability and development of DA-like neurons was increased when co-cultured with astrocytes as compared to using recombinant GDNF only. It was observed that the DA-like neurons that were co-cultured with astrocytes had more surviving cells, as well as a greater density of synapses. These results suggest that the methods utilized in the present study provide a more efficient and cost effective way of delivering GDNF to DA neurons.

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Poster

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Topic: C.03. Parkinson's Disease

Support: Swedish Research Council Grant 13482

Title: Regulation of JNK and c-Jun in experimental parkinsonism and L-DOPA-induced dyskinesia

Authors: *G. SPIGOLON[1], G. MENCATTELLI[1], S. VAN DER SPEK[1], M. FEYDER[1], M. TROPIANO[2], A. VERCELLI[2], G. FISONE[1];
[1]Karolinska Inst., Stockholm, Sweden; [2]Neurosci. Inst. Cavalieri Ottolenghi, Torino, Italy

Abstract: Parkinson's disease (PD) is characterized by the progressive degeneration of the nigrostriatal dopaminergic pathway and is commonly treated with the dopamine precursor, L-DOPA. Prolonged treatment with this standard pharmacotherapy results in the emergence of dystonic and choreic motor complications, termed L-DOPA-induced dyskinesia (LID). The identification of signaling abnormalities associated to LID is important for the design of more effective therapeutic strategies. Previous work showed that increased activity of the extracellular signal-regulated kinases and enhanced expression of the immediate early gene, FosB, are implicated in LID. FosB can promote gene transcription through the formation of heterodimers with members of the Jun protein family, such as c-Jun. Here, we used a mouse model of PD to study changes in c-Jun N-terminal kinase (JNK) and c-Jun associated with administration of L-DOPA and dyskinesia. We found that L-DOPA enhanced the phosphorylation of JNK in the striata of mice lesioned with the catecholaminergic toxin, 6-hydroxydopamine (6-OHDA). This effect was accompanied by a large increase in the expression and phosphorylation of c-Jun, a well-characterized substrate of JNK. The phosphorylation of c-Jun was restricted to a population of striatal medium spiny neurons, expressing dopamine D1 receptors (D1Rs). In agreement with this finding, the phosphorylation of JNK and c-Jun was also induced by administration of the selective D1R agonist, SKF81297. Interestingly, blockade of protein phosphatase-1 (PP-1), achieved with okadaic acid, increased the phosphorylation of JNK and c-Jun. Moreover, the ability of L-DOPA to promote c-Jun phosphorylation in dyskinetic mice was reduced by genetic inactivation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), an inhibitor of PP-1 implicated in LID. Altogether, these results suggest that abnormal activation of the JNK/c-Jun pathway, produced via the D1R/DARPP-32/PP-1 signaling cascade, is potentially involved in the development of LID.

Disclosures: G. Spigolon: None. **G. Mencattelli:** None. **S. Van Der Spek:** None. **M. Feyder:** None. **M. Tropiano:** None. **A. Vercelli:** None. **G. Fisone:** None.

Poster

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Topic: C.03. Parkinson's Disease

Support: CIHR

Title: D1R-mediated modulation of synaptic transmission in the bed nucleus of the stria terminalis of 6-OHDA lesioned rats with L-DOPA-induced dyskinesia

Authors: *C. DI PROSPERO[1], M. BASTIDE[2], E. R. HAWKEN[1], M. H. NAUGHTON[1], C. NORMANDEAU[1], F. GEORGES[2], E. BEZARD[2], E. C. DUMONT[1];
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Abstract: The gold standard treatment for Parkinson's disease (PD), L-3,4-dihydroxyphenylalanine (L-DOPA), results in involuntary movements referred to as L-DOPA-induced dyskinesia (LID). The mechanisms of LIDs are poorly known and there is no current strategy to efficiently prevent or control LIDs. Recent evidence has found that the expression of several immediate early genes is positively correlated with LID severity in a basal forebrain structure, the bed nucleus of the stria terminalis (BNST). Furthermore, evidence shows that dopamine D1-like receptors (D1Rs)- dependent overexpression of IEGs in the BNST may contribute to this phenomenon. Therefore this study aimed to examine how a D1R agonist modulates neuronal activity in the BNST of rats with and without LIDs using *in vitro* electrophysiology. Male Sprague Dawley rats (N=48) surgically received unilateral 6-OHDA lesions (2.5 µl at 3µg/µl). Three weeks post-op if the lesions produce PD symptoms measured by a stepping test, rats received daily injections of the vehicle Benserazide (15mg/kg, i.p.) or L-DOPA (6mg/kg, i.p.) in Benserazide. On the 10th injection day, rats were assessed for the severity of three subtypes of dyskinesia. Rats were then euthanized one hour post injection and whole cell patch clamping was done in both the juxtacapsular (jx) and oval (ov) areas of the BNST. L-DOPA treatment was not found to alter strength at excitatory synapses in the BNSTov or jx as measured by AMPA/NMDA ratios. However, we found a significant enhancement in D1 modulation of GABAa-mediated inhibitory synaptic transmission in the BNSTov of 6-OHDA-lesioned rats treated with L-DOPA vs the Benserazide controls. Furthermore, preliminary results suggest this is also the case in the BNSTjx. In addition, preliminary studies of D1 modulation of AMPA-mediated transmission suggest there is an enhancement in the L-DOPA rats vs. the Benserazide controls in the BNSTov but not BNSTjx. These data demonstrates intriguing extra-striatal neurophysiological traces of LID in the 6-OHDA-lesioned rat.

Disclosures: **C. Di Prospero:** None. **M. Bastide:** None. **E.R. Hawken:** None. **M.H. Naughton:** None. **C. Normandeau:** None. **F. Georges:** None. **E. Bezard:** None. **E.C. Dumont:** None.

Poster

693. Parkinson's Disease: Cellular Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 693.27/D42

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation Innovation Grant

Title: Generation of cell line models using the Zinc Finger Nuclease (ZFN) to preserve native regulatory elements for efficient high-throughput drug screenings

Authors: ***D. P. HUYNH**, W. DENSITHONG, S. PAUL, K. K. K. THAI, S. M. PULST; Dept Neurol, Univ. Utah, SALT LAKE CTY, UT

Abstract: Elevated levels of misfolded α-synuclein cause dopaminergic neuronal loss in Parkinson Diseases. Misfolded α-synuclein clearance and HADC inhibitors treatment were neuroprotective to dopaminergic neurons in PD animal and cell models suggesting that manipulating transcriptional gene expression and histone deacytylation can be used to prevent dopaminergic neuronal death in PD patients. However, there is no cell line model that has the entire SNCA regulatory elements for identifying specific HADC inhibitors and compounds that act on the SNCA regulatory elements. We aimed to produce cell lines that express either α-synuclein-luciferase or α-synuclein-GFP for high-throughput drug screenings for compounds that regulate SNCA histone deacytylation or transcriptional functions. Methods: We constructed a pair of ZFN-FokI and donor plasmids consisting of a GFP or Luciferase gene flanked by ~800 bp sequences up- and downstream of the ZFN-FokI cleaved site of the SNCA gene. The ZFN-FokI and donor plasmids were cotransfected into growing SH-SHY5Y cells, selected by 10 ug/ml puromycin, and confirmed by RT-PCR, Western blots, and bafilomycin A1 treatment. Results: Two SH-SY5Y cell lines, the SNCA-GFP and SNCA-Luc, were generated. RT-PCR confirmed that the marker gene was located at the desired site. Western blots using anti-GFP, anti-α-synuclein, and anti-luciferase antibodies confirmed that both the SNCA-GFP and SNCA-Luc cell lines produced α-synuclein-GFP and A-synuclein-luciferase fusion protein, respectively. No evidence of nonspecific donor DNA integration was observed. Treatment of bafilomycin A1, a lysosomal inhibitor, significantly increased the levels of luciferase of the SNCA-Luc cell line.

Conclusions: Using the ZFN method, we have generated two cell lines which will be useful in high-throughput drug screenings to identify compounds that can inhibit the elevation or expression of α-synuclein. These cell lines provide unique tools for drug screens as they include human SCNA regulatory control regions in promoters, introns, and even distant sites that potentially interact through chromatin loops.

Disclosures: D.P. Huynh: None. **W. Densithong:** None. **S. Paul:** None. **K.K.K. Thai:** None. **S.M. Pulst:** None.

Poster

694. Parkinson's Disease

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Title: Tyrosine hydroxylase as a genetic contributor to parkinson's disease progression

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Abstract: Parkinson's disease (PD) pathology involves the loss of nigrostriatal dopamine neurons that are marked by the selective expression of the enzyme tyrosine hydroxylase (TH). TH catalyzes the first and rate-limiting enzyme in catecholamine biosynthesis. The current study tested the hypothesis that genetic polymorphisms affecting dopamine synthesis and maintenance

of dopamine levels will alter the course and specific symptoms of PD. We sequenced 42 PD patients for a common polymorphism in the regulatory domain of TH (V81M). Subjects homozygous for this polymorphism displayed faster disease progression, as marked by the presence of the phenotype Freezing-of-Gait (FOG; p=0.0347 as determined by Mantel-Cox analysis with disease duration as a time marker), which presents as difficulty in initiating movement. A similar analysis performed with another common polymorphism in the dopamine metabolizing enzyme catechol-O-methyltransferase provided no such effect. In an effort to understand how the TH polymorphism may explain these clinical data, we investigated the effect of the V81M on TH function *in vitro*. Wildtype and V81M TH were expressed in E. coli and purified via affinity and size-exclusion chromatography. Kinetic characterization showed that enzyme stability was unchanged, and the Vmax and Km for the co-substrate BH4 were also unaffected. On the other hand, the Km for tyrosine was greater in V81M TH than in wild-type TH, suggesting that the enzyme activity may be decreased under normal physiological levels of tyrosine. These data suggest that genetic alterations in dopamine biosynthesis may affect the apparent progression of PD, as well as the phenotype of PD subjects carrying this polymorphism.

Disclosures: I. Tekin: None. **N. Carkaci-Salli:** None. **M.M. Lewis:** None. **R.B. Mailman:** None. **X. Huang:** None. **K.E. Vrana:** None.

Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: UFI 11/32

IT47-13

PI12/00613

Title: L-DOPA alters dopamine and serotonin release independently from electrophysiological changes in the dorsal raphe nucleus *in vivo*: Relevance to dyskinesia

Authors: *C. MIGUELEZ[1], S. NAVAILLES[2], C. DELAVILLE[2], L. MARQUIS[2], M. LAGIERE[2], A. BENAZZOUZ[2], L. UGEDO[1], P. DE DEURWAERDÈRE[2];

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Abstract: Numerous studies have pointed out serotonergic neurons as playing a pivotal role in the emergence of L-DOPA-induced dyskinesia (LID) in Parkinson's disease (PD. The nature of the release of dopamine (DA) and serotonin (5-HT) induced by L-DOPA together with the possible modifications induced in the dorsal raphe nucleus (DRN) are still unknown. To address the impact of L-DOPA at 5-HT cell bodies and terminals and its relevance to LID, we combined behavioural, electrophysiological and multi-site intracerebral microdialysis approaches in the 6-hydroxydopamine (6-OHDA) rat model of PD. We showed that acute L-DOPA treatment (6-12 mg/kg) did not modify DRN basal firing properties at odds with the multiple changes induced by L-DOPA (3-6-12-100 mg/kg) on 5-HT release monitored simultaneously in the striatum, prefrontal cortex (PFC), hippocampus (HIPP) and substantia nigra pars reticulata. Despite the induction of LID, chronic L-DOPA treatment (6 mg/kg/day) did not alter basal DRN neuronal activity or modify the sensitivity of 5-HT neurons to 5-HT1A receptor stimulation; however, it reduced the sensitivity of DRN activity to the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Using Ca2+-free medium or local application of the SSRI citalopram, we showed that L-DOPA (3-12 mg/kg) triggered both exocytotic (calcium-sensitive) and non-exocytotic (SERT-sensitive) mechanisms on *in vivo* 5-HT and DA releases whose proportions varied in the PFC and HIPP. These data show that L-DOPA-induced changes of 5-HT and DA releases reflect biochemical activities at 5-HT terminals independent from changes of 5-HT neuronal activity.

Disclosures: **C. Miguelez:** None. **S. Navailles:** None. **C. Delaville:** None. **L. Marquis:** None. **M. Lagiere:** None. **A. Benazzouz:** None. **L. Ugedo:** None. **P. De Deurwaerdère:** None.

Poster

694. Parkinson's Disease

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Program#/Poster#: 694.03/D45

Topic: C.03. Parkinson's Disease

Title: Extra-striatal dopaminergic activity and compensatory mechanisms in parkinsonian monkeys

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Abstract: The cardinal features of Parkinson's disease (PD) are associated with dopaminergic cell loss in the substantia nigra pars compacta (SNc). It is well demonstrated both in PD patients and animal models that parkinsonian motor features appear when SNc cell loss reaches 50-60% and striatal dopamine falls below 70-80%, suggesting some powerful compensatory mechanisms. Traditionally, the major pathophysiological emphasis in PD has resided in striatal DA deficit. However, the SNc has projections to other basal ganglia nuclei, and besides the thalamus, brainstem and cortex are dopaminergically innervated. To investigate the role and impact of modulating dopaminergic activity of extra-striatal basal ganglia nuclei, the selective D2LR agonist, Quinpirole (5.0μg/μL, 1.0-3.0μL), or the selective D2LR antagonist, Sulpiride (10μg/μL, 0.5-1.5μL), was infused locally into the somatomotor region of the subthalamic nucleus (STN) and the external segment of the globus pallidus (GPe) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (Macaca fascicularis) at three motor states (asymptomatic, recovered, and moderate parkinsonian states), and their behavioural effects were evaluated. The somatomotor regions of the STN and GPe were identified by extra-cellular neuronal recording and transient dyskinetic movements induced by local infusion of muscimol (1.0μg/μL, 1.0μL). The agonist infusion in the STN increased Kurlan score by 0.75, 1.63 and 2.5 points, while the antagonist infusion decreased it by 0.38, 0.5 and 1.25 points at asymptomatic, recovered and moderate parkinsonian states, respectively. On the other hand, their infusion in the GPe showed the opposite effects, that is, behavioural improvement with the agonist (decrease by 0.75, 0.5 and 2.0 points) and deterioration with the antagonist (increase by 0.88, 0.25 and 0.25 points of Kurlan score). These results suggest that dopaminergic activity of the GPe and STN can modulate motor activity once nigro-striatal dysfunction is present and that its functional role is not homogenous in the basal ganglia. Our results are in keeping with the hypothesis that early dopaminergic denervation in the STN plays a fundamental compensatory role in the pre-symptomatic state of PD.

Disclosures: H. Iwamuro: None. **I. Trigo-Damas:** None. **J. Obeso:** None.

Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant RO1.NS059600

Michael J. Fox Foundation

The Center for Development and Behavioral Neuroscience at Binghamton University

Title: Selective transporter blockade reveals differential contributions of monoamine transporters to L-DOPA's motor effects in hemi-parkinsonian rats

Authors: *M. CONTI, S. MEADOWS, M. MELIKHOV-SOSIN, E. NUSS, J. HALLMARK, N. VILCEUS, C. BISHOP;
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder typically characterized by nigro-striatal dopamine (DA) cell loss resulting in motor symptoms. DA replacement therapy with L-DOPA is the standard treatment for PD, but chronic treatment typically leads to the development of abnormal involuntary movements (AIMs) referred to as L-DOPA-induced dyskinesia (LID). Although LID mechanisms are poorly understood, they likely involve a complex interaction of the remaining DA system with the serotonin (5-HT) and norepinephrine (NE) systems. Non-specific monoamine reuptake inhibitors have been shown to enhance L-DOPA's anti-parkinsonian benefits without LID development, but the contribution of DA, 5-HT, and NE transporters (DAT, SERT, and NET, respectively) remain unclear. The current investigation sought to uncover the differential roles of DAT, SERT, and NET in L-DOPA's motor effects in unilateral 6-OHDA-lesioned Sprague-Dawley rats. After a 3 week recovery period, rats were primed with L-DOPA (6 mg/kg + benserazide 15 mg/kg; s.c.) and monitored for AIMs expression. Two subject cohorts were used to test DAT, SERT, and NET blockade via GBR-12909 (5, 10 mg/kg), citalopram (3, 5 mg/kg), and nisoxetine (5, 10 mg/kg), respectively, in counterbalanced within subjects designs. The first cohort tested transporter inhibition against LID by L-DOPA (3, 6 mg/kg). Findings indicated that only SERT blockade reduced LID while DAT and NET blockade mildly exacerbated LID expression. The second cohort investigated effects of transporter blockade in L-DOPA's therapeutic efficacy. No drastic changes were seen in L-DOPA's motor effects with citalopram, GBR12909, or nisoxetine pretreatment. The present results suggest that SERT mediates a unique and dominant mechanism underlying LID expression.

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Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant EB012864

Title: Modulation of mGluR4 and mGluR5 expression during Parkinson disease-like neurodegeneration

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Abstract: A loss of DA-mediated inhibition in the striatum results in excess excitatory transmission in Parkinson disease (PD). It is therefore possible that mGluR4 agonist or mGluR5 antagonist can improve PD symptoms directly. Furthermore, mGluR5 antagonists appear particularly promising for the treatment of levodopa-induced dyskinesias, a frequent, invalidating complication of DA replacement therapy that represents an abnormal form of synaptic plasticity. Finally, pharmacological manipulation of mGluRs could open up new modalities of neuroprotection for PD and other degenerative diseases of the nervous system, by decreasing excitotoxicity, modulating signaling pathways or, perhaps, through local translation of trophic factors. We have explored in 6-OHDA rat model of PD functional role of mGluR4 agonist and mGluR5 antagonists during progressive degeneration taking advantage of high sensitive positron emission tomographic imaging (PET) and our recently developed novel specific radiopharmaceuticals [11C]ML128 (N-(4-Chloro-3-[11C]methoxyphenyl)-2-picolinamide) for mGluR4 and [18F]FPEB (3-fluoro-5-[(pyridin-3-yl)ethynyl] benzonitrile) for mGluR5. In these studies we found that a unilateral nigral administration of 6-OHDA induced instantly decrease of dopamine transporter function in the striatum by 71± 5% followed by progressive degeneration of 0.12%±0.01 per month as compared to the control side. Longitudinal imaging of mGluR4 and mGluR5 showed that presynaptically expressed mGluR4 was enhanced in the striatum on the lesioned side as an immediate response to 6-OHDA administration while mGluR5 expression at that time had decreased. However, during the following progressive degeneration these changes decreased resulting an inverse correlation between mGluR4 and mGluR5 expression during striatal degeneration. mGluR4 expression was also enhanced in the hippocampus as an immediate response for 6-OHDA lesioning but declined during progressive degeneration. Interestingly progressive degeneration did not effect on mGluR5 expression which was enhanced about 4.47±0.69% during the whole follow up time of 15 months. During the progressive degeneration on the lesion (left) side regions there was no observable change of dopamine transporter function or expression of mGluR4 or mGluR5 on the control (right) side regions.

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Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: France Parkinson

Title: Impulsivity decreases dopaminergic neuronal vulnerability and inhibitory control following nigral neurodegeneration and dopamine replacement therapy

Authors: S. ANSQUER[1], M. ENGELN[2], E. DUGAST[1], N. DUTHEIL[2], E. BEZARD[2], D. BELIN[1], *P.-O. FERNAGUT[3];
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Abstract: Impulse control disorders are debilitating non-motor side effects of dopamine replacement therapy in Parkinson's disease (PD). The respective contributions of impulsivity, dopaminergic neurodegeneration and dopamine replacement therapy in the occurrence of dopamine agonist-induced impulse control disorders in PD are unknown. To identify how each of these factors may interact, we performed a longitudinal study in rats, not only investigating the role of multidimensional aspects of impulsive trait in the vulnerability to develop dopaminergic neuronal loss after overexpression of alpha-synuclein, but also the role of dopamine replacement therapy in increased impulsive state, that have been linked with higher level of compulsivity. inhibitory control performances were assessed using differential reinforcement of low rate of responding (DRL) and fixed consecutive number (FCN) procedures. Rats were ranked as high impulsive (HI), Intermediate (Int) or low impulsive (LI) according to their Z-scores. Assessment of DRL and FCN performances was then performed following viral-mediated overexpression of alpha-synuclein in the substantia nigra and after the administration of L-Dopa, apomorphine, or pramipexole. Alpha-synuclein-induced neurodegeneration significantly decreased DRL and FCN performances, with a greater effect in LI rats. While apomorphine and L-Dopa had little impact on DRL and FCN tasks in sham and lesioned rats,

pramipexole considerably affected DRL and FCN performances in both groups. There was a greater effect of pramipexole in HI lesioned rats in the DRL task. Histopathological analysis revealed that HI rats were less sensitive than their LI and Int counterparts to alpha-synuclein-induced neurodegeneration. These results indicate that impulsivity is associated with a differential vulnerability to alpha-synuclein-induced nigral dopaminergic neurodegeneration. While dopaminergic neurodegeneration impairs inhibitory control, an interaction between impulsivity, dopaminergic loss and dopamine replacement therapy underlies the deleterious effects of pramipexole on inhibitory control.

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Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: DA019486

Title: L-dopa induced decreases in serotonin neurons within the dorsal raphe nucleus are prevented by ascorbic acid

Authors: ***B. J. STANSLEY**, B. K. YAMAMOTO;
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Abstract: L-dopa is the precursor to dopamine and has become the mainstay therapeutic treatment for Parkinson’s disease (PD). High amounts of L-dopa are administered to recover motor function in PD patients; however, drug efficacy decreases over time and debilitating side effects such as dyskinesia, mood disorders, and hallucinations occur. The therapeutic effect and some of the side effects of L-dopa has been credited to serotonin (5-HT) neurons. Several studies have demonstrated the ability for 5-HT neurons to uptake L-dopa, decarboxylate it to dopamine, and exocytose dopamine in an impulse dependant manner. Further, studies in rats have found that chronic L-dopa treatment decreases forebrain 5-HT tissue content, suggesting that 5-HT neuron function is compromised. Furthermore, accumulation and degradation of dopamine within 5-HTergic cells results in oxidative stress and apoptosis in-vitro. Given these findings, it was hypothesized that chronic L-dopa treatment decreases 5-HT neurons and 5-HT tissue content

in the dorsal raphe nucleus (DRN) in an oxidative dependent manner. Rats were treated chronically with L-dopa (6 mg/kg; twice daily) for 10 days and killed 48 hrs after the last injection to investigate the effects on 5-HT neurons and 5-HT content in the DRN. For 5-HT neuron counts, coronal slices of the DRN were imaged by confocal microscopy, and cells positive for tryptophan hydroxylase and neuronal nuclei protein immunoreactivity were counted. Results indicated that after 10 days of L-dopa treatment, 5-HT neurons decreased in the DRN by about 15% ($t=2.325$, $p<0.05$). This effect was more pronounced in the caudal-dorsal sub-region of the DRN (↓28%) ($t=2.618$, $p<0.05$). Co-treatment with the anti-oxidant ascorbic acid (400 mg/kg) prevented the L-dopa induced decreases in 5-HT neurons within the DRN ($q=3.19$, $p<0.05$), suggesting that the L-dopa induced decreases in 5-HT neurons are oxidation dependant. Furthermore, 5-HT content of the DRN was measured by HPLC-EC, and was found to be decreased significantly by L-dopa treatment ($t=2.48$, $p<0.05$). Experiments examining ascorbic acid effects on L-dopa induced 5-HT tissue content decreases within the DRN are in progress. Future studies will examine the specific regional vulnerability of 5-HT neurons to L-dopa.

Disclosures: B.J. Stansley: None. **B.K. Yamamoto:** None.

Poster

694. Parkinson's Disease

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Support: Agence Nationale de la Recherche

Fondation de France

Université Lyon 1

Title: Acute MDMA intoxication abolishes L-DOPA-induced dyskinesia and behavioral hyperactivity in MPTP monkey model of Parkinson’s disease

Authors: *M. BEAUDOIN-GOBERT[1,2], E. MÉTÉREAU[1,2], J. EPINAT[1,2], S. NEUMANE[1,2], S. DUPERRIER[1,2], F. LIGER[3], C. TOURVIELLE[3], D. LE BARS[2,3], L. TREMBLAY[1,2], V. SGAMBATO-FAURE[1,2];
[1]Ctr. De Neurosci. Cognitive CNRS-UMR 5229, Bron, France; [2]Univ. Lyon 1, Lyon, France; [3]CERMEP - Imagerie du vivant, Lyon, France

Abstract: Convergent studies performed in humans have underlined an involvement of the serotonergic (5-HT) system in Parkinson's disease (PD). Correlations were evidenced between alterations of the 5-HT system and the expression of motor (tremor, dyskinesia) symptoms. Moreover, animal studies performed by our team evidenced an increase of striatal dopamine (DA) and serotonin (5-HT) in the striatum of parkinsonian monkeys recovering from their motor symptoms, suggesting that 5-HT could participate to compensatory mechanisms. To investigate the involvement of the 5-HT system in the expression of parkinsonian symptoms and behavioral effects induced by L-DOPA, the main symptomatic treatment for PD, we developed a monkey model exhibiting a double DA/5-HT lesion due to sequential use of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) followed by 3,4-methylenedioxy-N-methamphetamine (MDMA or Ecstasy). Monkeys received a chronic treatment of L-DOPA before and after MDMA. Depending on the mode of MPTP administration (acute or progressive), macaca fascicularis monkeys exhibited stable or transient motor symptoms and were therefore divided into stable or recovered groups. MDMA lesion did not evoke reappearance or worsening of tremor and akinesia/bradykinesia parkinsonian symptoms, suggesting that the 5-HT system does not play a compensatory role. But the 5-HT lesion counteracted the expression of rigidity when present in stable monkeys and favored it in recovered monkeys. Before MDMA, the long-term administration of L-DOPA evoked severe dyskinesia in stable monkeys and behavioral hyperactivity in recovered ones. Interestingly, both responses were abolished after MDMA. Plastic changes occurring in response to L-DOPA both before and after MDMA were investigated longitudinally by PET (positron emission tomography) imaging using in particular [11C]-PE2I and [11C]-DASB, respective ligands of the DA and 5-HT transporters. Those results were then confronted to post-mortem analysis performed by immunohistochemistry against DeltaFosB, a marker for dyskinesia, and DA and 5-HT markers. Our results highlight a role of 5-HT system in L-DOPA-induced dyskinesia and behavioral hyperactivity but refute the hypothesis that the 5-HT is involved in motor compensatory mechanisms.

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Poster

694. Parkinson's Disease

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Title: Regional deficits in neurotransmitter systems in demented Parkinson disease

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Neurol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: The development of dementia in PD is accompanied by neocortical α-syn deposition in Lewy bodies and Lewy neurites. Post-mortem studies in PD also demonstrate neuronal loss and deposition of aggregated α-syn in multiple subcortical nuclei including substantia nigra pars compacta (dopaminergic), dorsal raphe nuclei (serotonergic), and locus coeruleus (noradrenergic). Since these subcortical nuclei project to multiple brain regions, we aimed to determine whether dementia in PD is accompanied by regional deficits in neurotransmitter systems. We quantified dopaminergic, serotonergic and noradrenergic neurotransmitter systems in eight brain regions from 15 demented PD and 6 age-matched neurologically normal controls. HPLC and ELISA were used to quantify neurotransmitters and transporters, respectively. Preliminary data revealed that the levels of dopamine (DA) and dopamine transporter (DAT) were significantly lower in caudate (DA: $P = 0.001$, DAT: $P = 0.003$) and amygdala (DA: $P = 0.001$, DAT: $P = 0.001$) in PD when compared to controls; the levels of DAT were also lower in PD compared to controls for inferior parietal cortex ($P = 0.001$), visual association cortex ($P = 0.003$), precuneus ($P = 0.001$), amygdala ($P = 0.001$) and hippocampus ($P = 0.002$). Serotonin (5HT) levels were significantly lower in PD compared to controls for middle frontal cortex ($P = 0.001$), anterior cingulate cortex ($P = 0.01$), caudate ($P = 0.003$), inferior parietal cortex ($P = 0.0005$) and visual association cortex ($P = 0.003$). Similar to 5HT, serotonin transporter levels were significantly lower in PD compared to controls for inferior parietal cortex ($P = 0.0005$) and visual association cortex ($P = 0.001$), in addition to being lower for middle frontal cortex ($P = 0.0005$) and caudate ($P = 0.01$). Nor epinephrine levels were significantly lower in PD when compared to controls for all brain regions; caudate ($P = 0.001$), anterior cingulate cortex ($P = 0.0005$), mid-frontal cortex ($P = 0.002$), inferior parietal cortex ($P = 0.001$), precuneus ($P = 0.007$), visual association cortex ($P = 0.001$), amygdala ($P = 0.002$) and hippocampus ($P = 0.01$). These results demonstrate that PD causes regional deficits in innervation from subcortical nuclei producing dopamine, serotonin and norepinephrine. Enrollment of additional participants in the prospective study will be helpful to determine whether specific regional neurotransmitter changes correspond with specific ante-mortem cognitive phenotypes.

Disclosures: C. Buddhala: None. **M.C. Campbell:** None. **S.K. Loftin:** None. **P.T. Kotzbauer:** None. **J.S. Perlmutter:** None.

Poster

694. Parkinson's Disease

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 694.10/D52

Topic: C.03. Parkinson's Disease

Support: Walter S. and Lucienne Driskill Foundation

Title: Transplanting human dopamine neurons derived from ES cells and iPS cells into rat striatum

Authors: W. ZHOU[1], Y.-M. LEE[2], K. B. BJUGSTAD[2], M. FERREYROS[2], *C. R. FREED[3]; [1]Div. of Clin. Pharmacology, Dept. of Med., Univ. of Colorado Sch. of Med., AURORA, CO; [2]Div. of Clin. Pharmacology, Dept. of Med., [3]Div. of Clin. Pharmacol., Univ. Colorado Sch. of Med., AURORA, CO

Abstract: The most effective treatment for Parkinson's disease is the drug L-DOPA, but its short half-life and unpredictable response after many years of therapy has led to the search for new treatments. Our prior experience with human fetal dopamine cell transplantation has shown that transplants placed bilaterally and simultaneously into putamen without immunosuppression can survive for the life of the patient and can replicate the effects of L-DOPA. In the first double-blind, placebo controlled clinical trial, we showed that transplants produced significant improvement in UPDRS motor "off" scores with the average clinical response being 60% of the best effects of L-DOPA by 3 years after transplant (n=34) regardless of age and without immunosuppression. Because acquiring human fetal tissue is difficult, we have differentiated human embryonic stem cells (ES cells) and human induced pluripotent stem cells (iPS cells) to dopamine neurons using a variety of differentiation conditions. Transplantation into a single striatal site in female NIH nude rats with unilateral 6OHDA lesions was performed. Groups of 5 rats received grafts of iPS or ES cell-derived dopamine neurons with or without co-transplants of astrocytes, hydrogels, and hydrogels containing the growth factors GDNF, BDNF, and bFGF. Transplant growth was followed with monthly tests of apomorphine and methamphetamine-induced circling. 16 weeks after transplant, most groups showed significant reduction in apomorphine and methamphetamine circling compared to sham operated controls. Animals were sacrificed and dopamine neuron survival assessed with immunohistochemical staining for tyrosine hydroxylase and other dopaminergic markers. Results showed dopamine neuron survival was markedly different between treatment groups depending on the differentiation method and the co-transplant composition. We conclude that optimizing both *in vitro*

differentiation and co-transplant survival factors is important for transplantation of stem cell-derived dopamine neurons into patients with Parkinson's disease.

Disclosures: W. Zhou: None. **Y. Lee:** None. **K.B. Bjugstad:** None. **M. Ferreyros:** None. **C.R. Freed:** None.

Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant MH085074

R01EB016407

Title: An acute pharmacological approach for the study of information transmission through the basal ganglia

Authors: *K. M. LAMBERT, J. A. WHITE, A. D. DORVAL;
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Abstract: Deep brain stimulation (DBS) is an effective neurosurgical intervention for several neurological disorders of the basal ganglia, including Parkinson's disease and dystonia. The therapeutic mechanisms of DBS remain unclear, as evidence mounts that DBS induces neurophysiological changes that do not conform to traditional neuronal-rate theories of symptom severity. To improve these models of basal ganglia processing, we employ a pharmacological approach to isolate the information propagating through basal ganglia pathways from the firing rates of the carrier neurons. Dopamine-receiving medium spiny neurons (MSNs) in the striatum initiate two separate pathways through basal ganglia: D1 receptor-dominant MSNs begin the action-promoting, direct pathway; D2 receptor-dominant MSNs begin the action-suppressing, indirect pathway. D1 activation (e.g., by dopamine) increases the firing rate of D1 MSNs, inhibiting downstream neurons in the direct pathway. D2 activation (by dopamine) decreases the firing rate of D2 MSNs, disinhibiting downstream neurons in the indirect pathway. In this study, we selectively block each pathway, using either the D1 antagonist SCH 23390, or the D2 agonist quinpirole. These interventions should reduce activity in the respective pathways, leaving information through the opposite pathway to be quantified in isolation. We have verified our

ability to elicit the expected behavioral results from the selective blocking of each pathway. Unilateral and bilateral injections of SCH 23390 into striatum result in a cataleptic state, assessed as less time spent moving and less distance traveled in the hours following injection, relative to rest; unilateral and bilateral injections of quinpirole result in hyperactivity assessed as more time spent moving, and a greater distance traveled. The behavioral effects of each drug are temporary, and saline injections on following days lead to activity levels that match control conditions. Therefore, intrastriatal injections of the selective D1 antagonist SCH 23390 or the selective D2 agonist quinpirole may be used as a mechanism to block information transmission discerningly through the pathways of the basal ganglia.

Disclosures: K.M. Lambert: None. **J.A. White:** None. **A.D. Dorval:** None.

Poster

694. Parkinson's Disease

Location: Halls A-C

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Program#/Poster#: 694.12/D54

Topic: C.03. Parkinson's Disease

Title: Role of glucose transporter1 (Glut-1) in an MPTP- induced mouse model of Parkinson's disease

Authors: *S. SARKAR[1], J. RAYMICK[2], L. SCHMUED[3], S. CHIGURUPATI[3], M. G. PAULE[3];
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Abstract: Glucose is an important fuel for the brain and its transport into the brain is generally mediated by one or more members of the glucose transporter (GLUT) family. Glut-1 is expressed at high concentration in the capillary endothelium of the blood-brain barrier and plays an important role in transferring glucose across that barrier. The level of Glut-1 expression is tightly regulated in concert with metabolic demand and regional rates of glucose utilization. It is well known that MPTP, a dopaminergic neurotoxicant, inactivates complex I of the electron transfer chain in mitochondria and ultimately leads to neuronal degeneration mimicking PD in humans. Mitochondrial dysfunction also perturbs energy metabolism which in turn contributes to PD development. Although mitochondrial dysfunction and energy deficits appear to correlate with the development of PD symptoms, it is unknown whether these alterations in metabolic capability affect the expression of Glut-1 in the brain, particularly in the striatum and substantia

nigra. Mouse models of PD employing acute, sub-chronic and chronic exposure to MPTP were used to determine the extent to which brain Glut-1 expression may be affected as PD symptoms develop. The most significant changes in the diameter and intensity of Glut-1 immunoreactive endothelial cells were seen following acute or chronic MPTP treatment. Animals treated sub-chronically with MPTP exhibited moderate changes in the diameter and intensity of Glut-1 expressing endothelial cells. To confirm that these Glut-1 immunoreactive structures were endothelial cells, we co-labeled them with PECAM that labels only endothelial cells. Complete co-localization was observed between PECAM and Glut-1. Double immunolabeling for tyrosine hydroxylase (a rate limiting enzyme for dopamine synthesis) and Glut-1 revealed that TH loss/degeneration in the striatum correlates with reductions in Glut-1 expression. This study suggests the importance of endothelial cells in glucose transport and impairment of glucose transport during development of PD pathology.

Disclosures: S. Sarkar: None. **J. Raymick:** None. **L. Schmued:** None. **S. Chigurupati:** None. **M.G. Paule:** None.

Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

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FORP-USP

FMRP-USP

CAPES

CNPq

Title: Effects of neuronal nitric oxide synthase (nNOS) inhibition on L-DOPA-induced striatal ERK1/2 phosphorylation in the 6-OHDA rat model of Parkinson's disease

Authors: *F. E. PADOVAN NETO, E. DEL BEL;
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Abstract: The interaction between abnormal striatal plasticity and changes in non-dopaminergic transmitter systems is thought to contribute to the pathophysiology of L-DOPA-induced dyskinesia (LID). The role of the atypical neurotransmitter nitric oxide (NO) in LID was demonstrated by the anti-dyskinetic properties of NO synthase (NOS) inhibitors. A putative molecular marker of LID is the abnormally high levels of phosphorylated extracellular-regulated kinase 1/2 (p-ERK1/2) within the striatum. ERK1/2 mediates changes in gene expression via phosphorylation of histone kinase proteins and activation of nuclear transcription factors. This study aimed to analyze the anti-dyskinetic effect of the neuronal NOS inhibitor 7-nitrondazole (7-NI) on neuronal NOS (nNOS) - soluble guanylyl cyclase (sGC) pathway and on the state of phosphorylation of ERK1/2 in the dorsal striatum. 6-OHDA-lesioned rats were divided into four treatment groups which received the combination of the following treatments (chronically, once a day, for 21 days, n=5-9/group): (i) vehicle (2 ml/kg) + saline (2 ml/kg), (ii) 7-NI (30 mg/kg) + saline (2 ml/kg), (iii) vehicle (2 ml/kg) + L-DOPA (10 mg/kg), 7-NI (30 mg/kg) + L-DOPA (10 mg/kg). A group of Sham-operated animas (n=6) was also included in the study. Rats were examined behaviorally for a rat dyskinesia scale and for the stepping test. Western blot analysis was performed in total homogenates of the dorsal striatum to analyze the expression of nNOS, sGC and p-ERK1/2. Chronic administration of L-DOPA reversed akinesia and induced the appearance of axial, limb, orofacial and locomotive dyskinesia in 6-OHDA-lesioned rats with >90% of dopaminergic cell loss in substantia nigra compacta. LID was associated with an up-regulation of nNOS, sGC and p-ERK proteins in the dorsal ipsilateral striatum. Prolonged administration of 7-NI blocked the manifestation of LID by chronic L-DOPA treatment without interfering with the antiparkinsonian effect of L-DOPA as demonstrated by the stepping test. Chronic 7-NI also inhibited L-DOPA-induced up-regulation of nNOS, sGC and p-ERK proteins in the dorsal ipsilateral striatum. The manipulation of NO system with nNOS inhibitors as an anti-dyskinetic prophylactic approach was effective in normalizing the up-regulation of nNOS, sGC and p-ERK1/2 protein expression in ipsilateral dorsal striatum. The anti-dyskinetic effects of NOS inhibitors in striatal ERK1/2 expression may contribute to the understanding of this newly non-dopaminergic therapy for the management of LID.

Disclosures: **F.E. Padovan Neto:** None. **E. Del Bel:** None.

Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NSF Grant 0823358

Title: Dopamine toxicity and oxidative stress in zebrafish larvae as a model of Parkinson's disease neuropathology

Authors: *S. J. STEDNITZ, S. SHELTON, T. SHEN, D. BLACK, N. LAPOLLA, B. FRESHNER, K. HARTSUYKER, E. GAHTAN;
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Abstract: Dopamine signaling is conserved across most animals and disruption of dopamine is implicated several neurological disorders, including Parkinson's disease (PD). The primary neuropathology in PD involves the death of dopaminergic neurons in the substantia nigra (SN), an anatomical region of the brain implicated in voluntary motor control. After release, dopamine is transported back into cells by the dopamine transporter (DAT), a membrane-spanning protein expressed in many dopaminergic neurons and glial cells. Intracellular dopamine is metabolized by monoamine oxidase (MAO), producing dopamine aldehyde (DOPAL), a potent oxidative molecule. Although DOPAL is normally short-lived inside cells, it may have toxic effects in vulnerable neurons by causing oxidative damage to mitochondria. We examined the effects of dopamine metabolism on dopaminergic neurons and motor behavior in transgenic zebrafish larvae in which all neurons expressing the DAT are fluorescent (dat:EGFP zebrafish). Larvae were exposed to L-DOPA alone (1mM, bath applied) in order to increase levels of DOPAL, or to L-DOPA together with the MAO inhibitor, rasagiline (100uM), to prevent DOPAL generation. Confocal microscope imaging of dat:EGFP neurons within the pretectum and ventral diencephalon revealed a reduction in the number and fluorescence intensity of dopaminergic cells due to L-DOPA, and this was effect was reduced by MAO inhibition. Spontaneous locomotor behavior in L-DOPA treated animals was depressed 24 hours after L-DOPA treatment, while visually-evoked startle response rates and latencies were unaffected. Although rasagiline appeared to prevent some of the L-DOPA mediated cell loss, locomotor impairment was actually greater in rasagiline treated larvae, likely due to elevated serotonin. These results suggest that L-DOPA treatment may indirectly contribute to dopamine neuron loss, and that adjunctive treatment with MAO-B inhibitors may delay the loss of dopaminergic neurons in PD.

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Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Lundbeck Foundation

Title: Computational modeling of striatal denervation: Deteriation of dopamine signaling by three mechanisms

Authors: *J. K. DREYER;
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Abstract: Parkinson's disease (PD) is a common age-related neurological disease. The classical symptoms include rigidity, bradykinesia, resting tremor and cognitive symptoms. The hallmark of PD is progressive degeneration of dopamine (DA) neurons. However, there is no fully consistent mechanism accounting for progression, onset of symptoms, and medication of PD. For example, even though D2 receptor agonists alleviate motor symptoms, D2 receptor binding as measured with PET does not correlate with disease duration or severity of symptoms in early and intermediate PD. Likewise, animal models often have normal DA levels even with substantial striatal DA depletion, but nevertheless substantial changes in post synaptic D1 and D2 receptor signaling cascades occurs with mild denervation. A coherent mechanistic account of PD is thus complicated because the exact delineation between biophysical, physiological, and compensatory changes in the DA signal is unknown. Here I conduct a theoretical analysis based on biophysical models of DA volume transmission at different stages of denervation. The analysis predicts that even with optimal post synaptic compensation malfunction develops by at least three independent mechanisms: 1) Denervation increases the time constant of DA dynamics. Thus eventually a mismatch of timescales between DA dynamics and the time scale of bursts and pauses in firing patterns arises. This phenomenon can occur with relatively low denervation (50%-80%), and affects mainly signaling by high affinity DA D2 receptors ($D2^{high}$). 2) Denervation exceeding 80% also compromises signal to noise ratio in the DA signal. In combination with super sensitive post synaptic pathways this leads to a persistent pathological signal affecting mainly $D2^{high}$ receptor regulated pathways. 3) With sufficient DA loss, some areas end up fully depleted. Inside such areas DA levels will be permanently low extracellular DA if their macroscopic size exceeds approximately 500 μm. This provides a mechanistic account analysis compatible with clinical observations such as normal D2 binding in patients with early stage PD and late onset of symptoms. Furthermore it accounts for the effect, breakdown, and side-effects of L-Dopa therapy and provides a general scheme compensatory regulation of post synaptic signals.

Disclosures: **J.K. Dreyer:** None.

Poster

694. Parkinson's Disease

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Support: Centre National de la Recherche Scientifique

Université de Strasbourg

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Fondation pour la Recherche Médicale

NIH (MH067937)

The Michael J. Fox Foundation for Parkinson's Research

Title: Control of the nigrostriatal dopamine neurons, of motor performance and of motor skill learning by the tail of the ventral tegmental area

Authors: *M.-J. SANCHEZ-CATALAN[1], R. BOURDY[1,2], J. BALCITA-PEDICINO[3], J. KAUFLING[1,4], M.-J. FREUND-MERCIER[1,2], P. VEINANTE[1,2], S. SESACK[3], F. GEORGES[4,5], M. BARROT[1];
[1]INCI -CNRS UPR3212, Strasbourg, France; [2]Univ. de Strasbourg, Strasbourg, France; [3]Univ. of Pittsburgh, Pittsburgh, PA; [4]Interdisciplinary Inst. for Neuroscience- CNRS UMR 5297, Bordeaux, France; [5]Univ. de Bordeaux, Bordeaux, France

Abstract: Midbrain dopamine neurons are implicated in various psychiatric and neurological disorders. The GABAergic tail of the ventral tegmental area (tVTA), also named the rostromedial tegmental nucleus (RMTg), displays dense projections to the midbrain and exerts electrophysiological control over dopamine cells of the VTA. However, the influence of the tVTA on the nigrostriatal pathway, from the substantia nigra pars compacta (SNc) to the dorsal striatum, and on related functions remains to be addressed. The present poster highlights the role played by the tVTA as a GABA brake for the nigrostriatal system, demonstrating a critical influence over motor functions. Using neuroanatomical approaches with tract-tracing and

electron microscopy, we evidence the presence of a tVTA-SNc-dorsal striatum pathway. Using *in vivo* electrophysiology (with electrical stimulation, chemical stimulation with glutamate, and chemical inhibition with muscimol), we prove that the tVTA is a major inhibitory control center for SNc dopamine cells. Using behavioral approaches (with rotation behavior after unilateral lesion, and rotarod test after bilateral lesion), we demonstrate that the tVTA controls motor coordination and motor skill learning. The motor enhancements observed after ablation of the tVTA are in this regard comparable to the doping properties of the performance enhancer amphetamine. These findings demonstrate that the tVTA is a major GABA brake for nigral dopamine systems and nigrostriatal functions, and they raise important questions about how the tVTA is integrated within basal ganglia circuitry. They also warrant further research on the tVTA's role in motor and dopamine-related pathological contexts such as Parkinson's disease.

Disclosures: M. Sanchez-Catalan: None. **R. Bourdy:** None. **J. Balcita-Pedicino:** None. **J. Kaufling:** None. **M. Freund-Mercier:** None. **P. Veinante:** None. **S. Sesack:** None. **F. Georges:** None. **M. Barrot:** None.

Poster

694. Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: CDBN

R01-N5059600

Title: L-DOPA-induced striatal norepinephrine efflux is associated with dyskinesia in hemi-parkinsonian rats

Authors: *J. A. GEORGE, C. Y. OSTOCK, A. A. GOLDENBERG, M. CONTI, C. BISHOP; Psychology, Binghamton Univ., Binghamton, NY

Abstract: L-DOPA remains the primary treatment for Parkinson's disease (PD). Unfortunately, its therapeutic benefits are compromised by the development of abnormal involuntary movements (AIMs) known as L-DOPA-induced dyskinesia (LID). Although LID is often linked to L-DOPA-induced striatal dopamine (DA) fluctuations, recent research has also implicated a more causal role for norepinephrine (NE). Thus, the current study sought to characterize L-

DOPA-induced striatal DA and NE efflux, while simultaneously monitoring the behavioral effects of chronic L-DOPA treatment. First, adult male Sprague-dawley rats received either sham or unilateral 6-hydroxydopamine (6-OHDA) lesions of the left medial forebrain bundle (MFB) and microdialysis guide cannulae into the DA-lesioned striatum. Animals were then primed with L-DOPA (6 mg/kg, s.c.) for 2 weeks until consistent AIMs developed. Thereafter, L-DOPA-mediated striatal monoamine efflux was measured with microdialysis, and concurrent AIMs testing occurred to determine responsiveness to L-DOPA (3 or 6 mg/kg, s.c.). Not surprisingly, AIMs were only observed in DA-lesioned rats. Remarkably, however, L-DOPA-induced striatal NE efflux rather than DA efflux, corresponded more closely with dyskinesia severity in DA-lesioned animals. The current study implicates L-DOPA-induced striatal NE as an important factor in LID expression and demonstrates the importance of developing treatment strategies that may modulate both the NE and the DA system.

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Poster

694. Parkinson's Disease

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Title: Effect of a chronic treatment with L-DOPA and MPEP, an mGlu5 receptor antagonist, on basal ganglia serotonin markers of de novo parkinsonian monkeys

Authors: *N. MORIN, M. MORISSETTE, L. GRÉGOIRE, T. DI PAOLO;
Neurosci. Res. Unit, Laval Univ. Med. Ctr. (CHUL), Quebec City, QC, Canada

Abstract: In the long term, approximately 80% of Parkinson's disease (PD) patients treated with L-3,4-dihydroxyphenylalanine (L-DOPA) will develop abnormal involuntary movements including L-DOPA-induced dyskinesias (LID). Brain glutamate overactivity is well documented in PD and antiglutamatergic drugs are proposed to relieve PD symptoms and decrease LID. The effects of long-term treatment with these drugs are yet to be characterized. The objective of this

study was to investigate the long-term effect of the prototypal metabotropic glutamate 5 (mGlu5) receptor antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) with L-DOPA on basal ganglia serotonin transporter SERT as well as 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2A} receptor levels in monkeys lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP monkeys were treated for one month with L-DOPA and developed LID while those treated with L-DOPA and MPEP (10 mg/kg) developed significantly less LID. Normal controls and saline-treated MPTP monkeys were included for biochemical analysis. The MPTP lesion and experimental treatments left unchanged striatal 5-HT concentrations. MPTP lesion induced an increase of striatal 5-HIAA concentrations similar in all MPTP monkeys as compared to controls. [^{3}H]-8-OH-DPAT and [^{3}H]-citalopram specific binding levels to 5-HT_{1A} receptors and SERT respectively remained unchanged in the striatum and globus pallidus of all MPTP monkeys compared to controls and no difference was observed between groups of MPTP monkeys. [^{3}H]-ketanserin and [^{3}H]GR125743 specific bindings to striatal and pallidal 5-HT_{2A} and 5-HT_{1B} receptors respectively were only increased in L-DOPA-treated MPTP monkeys as compared to controls, saline and L-DOPA+MPEP MPTP monkeys and no difference between the latter groups was observed. Moreover, dyskinesias scores correlated positively with both [^{3}H]-ketanserin and [^{3}H]GR125743 specific binding levels. In conclusion, reduction of development of LID with MPEP was associated with lower striatal and pallidal 5-HT_{2A} and 5-HT_{1B} receptors showing that glutamate activity also affects serotoninergic markers. These results support the therapeutic use of mGlu5 receptor antagonisms in PD in order to prevent the development of LID.

Disclosures: **N. Morin:** None. **M. Morissette:** None. **L. Grégoire:** None. **T. Di Paolo:** None.

Poster

694. Parkinson's Disease

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Michael J. Fox Foundation

Title: Reliability and accuracy of positron emission tomography reference region models of the nigrostrial dopaminergic system

Authors: *J. S. PERLMUTTER, L. TIAN, Y. SU, S. LOFTIN, H. FLORES, S. MOERLEIN; Washington Univ. Sch. Med., SAINT LOUIS, MO

Abstract: A variety of noninvasive reference tissue models have been applied for analysis of three classes of radiotracers used with positron emission tomography (PET) for assessment of presynaptic nigrostriatal neurons, including 6-[18F]fluorodopa (FD for decarboxylase activity), [11C]dihydrotetrabenazine (DTBZ for vesicular monoamine transporter type 2 [VMAT2]), and 2β-[11C]carbomethoxy-3β-(4-fluorophenyl)tropane (CFT for dopamine transporter [DAT]). There have been relatively few studies comparing the reliability and accuracy of these methods. The aim of this study was to assess the reliability and accuracy of PET measures with FD, DTBZ and CFT using different reference region methods. 16 Monkeys had variable unilateral intracarotid doses (0 - 0.31 mg/kg) of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Each monkey had 1 baseline MRI scan, 2 baseline and 2 post-MPTP PET scans for each tracer. The 3 methods for FD were the Patlak graphical plot (Patlak), reference region-based Logan (Logan), and tissue ratio (TRM). The 8 analyses for DTBZ, and CFT were Logan, TRM, reference tissue (RTM), simplified reference tissue (SRTM), simplified reference tissuel 2 (SRTM2), original multilinear reference tissue (MRTMo), multilinear reference tissue (MRTM), and multilinear reference tissue 2 (MRTM2). To evaluate the reliability of estimated repeated binding variables for baseline and post-MPTP scans, test-retest variability (TRV), intraclass correlation coefficient (ICC; a relative measure of reliability), and standard error of measurement (SEM; an absolute measure of reliability) were calculated. To evaluate the accuracy of each tracer, striatal PET measures were compared to *in vitro* measures made with high performance liquid chromatography (HPLC) for dopamine concentration and autoradiography for Bmax of VMAT2 and DAT. The TRV, ICC, and SEM values for FD, DTBZ and CFT estimates show good reliability in baseline scans, but differed for the lesioned striatum among methods. For all tracers and all methods, striatal PET uptake variables post-MPTP correlated strongly with *in vitro* measures ($r > 0.88$, $p < 0.0005$). In conclusion, although the accuracy assessed for each tracer using the reference region methods was very good, the reliability of those methods varied between baseline and post-MPTP scans.

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Poster

694. Parkinson's Disease

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UNMC fellowship

Title: Increased tyrosine hydroxylase expression by GABAergic neurons in response to chronic dopamine replacement therapy

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Abstract: In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of dopaminergic neurodegeneration, we showed that chronic administration of L-3,4-dihydroxyphenylalanine (L-DOPA) or BL-1023 (an L-DOPA-GABA conjoined compound) for 35 days post-lesion development, improved cumulative rotatrod performance that correlated with increased numbers of tyrosine hydroxylase (TH) immunoreactive (TH+) neurons in the substantia nigra (SN) of MPTP-treated mice compared to those given no drug. Increased numbers of TH+ neurons were shown to be derived from a phenotypic shift of GABAergic neurons that upregulate TH. Stereological analysis of the ventral midbrain for neurons that express TH, a dopaminergic marker, or glutamic acid decarboxylase-67 (GAD67), a marker for GABAergic neurons, demonstrated that mice treated with MPTP and either L-DOPA or BL-1023 showed significant increases in the numbers of TH+GAD67+ neurons in the SN compared to controls. No significant nuclear incorporation of bromodeoxyuridine (BrdU) by TH+ neurons within the SN of mice treated with PBS, MPTP, L-DOPA, or BL-1023; however, BrdU incorporation was evident among TH+ neurons of the olfactory bulbs in untreated mice. Together these data indicated little or no neurogenic activity among TH+ neurons of the SN and suggested that increased number of nigral TH+ neurons following MPTP and drug treatment were not due to neurogenesis. More likely, these findings support the notion that GAD67+ neurons upregulate TH expression and effectively increase the numbers of TH+ neurons in response to MPTP-intoxication, dopaminergic neuron loss, and chronic dopamine-replacement drug administration. Preliminary quantitative PCR data using primary neural cultures isolated from the ventral midbrain of mice showed consistent levels of GAD67 gene expression indicating the presence of GABAergic neurons in our *in vitro* cultures. Furthermore, treating primary neurons with 10 μM L-DOPA or 1 μM nicotine, a compound inversely correlated with Parkinson's disease development, for 24 hours induced the expression of Pitx3, a transcription factor responsible for regulation of TH expression within the SN. Lack of expression of TH mRNA by *in vitro* cultures, likely due to the highly temporal regulation of TH expression, suggests a longer

exposure time to TH-inducing compounds is required for *in vitro* neuron culture. Together our *in vivo* and *in vitro* results provide probable mechanisms underlying upregulation of TH by GABAergic neurons along the nigrostriatal axis that may reflect putative compensatory or reparative responses to dopaminergic loss to increase dopamine production in a dopamine-depleted environment.

Disclosures: K. Anderson: None. **R. Mosley:** None. **A.M. Szlachetka:** None. **J.L. Hutter-Saunders:** None.

Poster

694. Parkinson's Disease

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 694.21/D63

Topic: C.03. Parkinson's Disease

Support: CONACYT México 152326

Title: Dopamine denervation disrupts CAMKII mediated cytoplasmic Ca2+ signaling via a reduction in calmodulin levels

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Abstract: In both the n. accumbens and in striatonigral projections increased Ca^{2+} entry suppresses dopamine D3receptor (D3R) responses via CAMKII activation. These inhibitory effects of high Ca^{2+} are blocked by the specific CAMKII antagonist KN-62. We used hemiparkinsonian rats produced by unilateral injections of 6-OHDA into the medial forebrain bundle to investigate the effects of dopamine dennervetion on CAMKII mediated modulation of cAMP production and K^{+}-depolarization induced [^{3}H] GABA release in the SNr. After lesioning, the inhibitory effects of CAMKII activation on D3R signaling were absent and the responses to D3R activation were not modified by blocking CAMKII with KN-62. Immunoblot studies showed that depolarization-induced CAMKII phosphorylation and CAMKII binding to the D3 receptor were absent in the lesioned tissues. Because calmodulin is a major modulator of CAMKII activity we determined whether denervation affects calmodulin expression using PCR

and immunoblot techniques. Both techniques showed that calmodulin expression was depressed in the lesioned side while CAMKII levels didn't appear to be modified. These results demonstrate that after dopamine cell loss, CAMKII modulation by cytoplasmic Ca^{2+} is disrupted. Since the responses of CAMKII to changes in cytoplasmic [Ca^{2+}] are determined by calmodulin activation we suggest that the loss of modulation by Ca^{2+} entry is produced by reduced calmodulin levels.

Disclosures: S. Albarrán-Bravo: None. **A. Avalos-Fuentes:** None. **S. Loya-Lopez:** None. **F. Paz-Bermudez:** None. **H. Cortes-Calleja:** None. **D. Erlij:** None. **J. Aceves:** None. **B. Florán Garduño:** None.

Poster

694. Parkinson's Disease

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: FAPESP

CNPq

CAPES

NAPNA

USP

Title: Haloperidol induced-extrapyramidal symptoms are increased in iNOS-knockout mice

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Abstract: Haloperidol is a widely prescribed antipsychotic that acts as a dopamine D2 receptor (D2) antagonist. The blocking of D2 by haloperidol has been mostly associated with antipsychotic therapeutic action. It is also associated with extrapyramidal symptoms (EPS), which are present in movement disorders such as akinesia, catalepsy, muscular rigidity, and involuntary eyelid closure. Recent studies suggest an involvement of nitric oxide synthase (NOS)

in dopaminergic supersensitivity. Inhibition of nNOS activity by NOS inhibitors in rodents have shown to mimic the action of antipsychotic drugs and attenuate animal locomotor activity, to decrease acute haloperidol-induced catalepsy, or to increase vacuous chewing movements after withdrawal from chronic haloperidol treatment. However, the role of inducible NOS (iNOS, NOS2) in the development of haloperidol-induced EPS has not been reported. In this investigation, male 6-8 wk-old C57BL/6 and iNOS-knockout (iNOS-KO) mice were treated with saline or haloperidol (5 mg/kg, i.p.). After 1, 2 and 4 hours, we detected catalepsy in both haloperidol-inoculated genotypes when compared to saline controls. Interestingly, in all evaluated time points, the duration of haloperidol-induced catalepsy was increased in iNOS-KO when compared to C57BL/6 mice. Moreover, the eyelid closure was visually scored (completely open or closed; one-quarter, half or three-quarters closed) after 1, 2 and 4 h post haloperidol inoculation. We found that haloperidol-induced palpebral closure was intensified progressively after treatment in both evaluated genotypes. However, the involuntary eyelid closure was more prominent in the iNOS-KO group. To measure rigidity, the time spent hanging from the side of a cage by the hind legs was analyzed 3h after treatment. Our results showed that haloperidol increased the grasping time only in iNOS-KO mice. Finally, we measured akinesia 3 h after inoculation by counting the number of forward steps taken with both forepaws while the mice were held by the tail. The haloperidol-induced akinesia was observed in both groups, however the number of steps was significantly diminished in iNOS-KO mice in comparison with C57BL/6 counterpart. In conclusion, this study suggests that mice deletion of iNOS is associated with enhanced haloperidol-induced EPS.

Disclosures: **F.M. Dias:** None. **G.H.D. Abreu:** None. **T.F. Zanin:** None. **E.A. Del Bel:** None.

Poster

694. Parkinson's Disease

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Support: FAPESP

CAPES

CNPq - Science Without Borders-Special Research Visitor

FORP-USP

FMRP-USP

NAPNA

Title: Ameliorating effects of toll-like-9 receptor (TLR9) deletion on 6-OHDA-induced dopaminergic denervation and astrocytic response in the striatum of hemiparkinsonian mice

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Abstract: In mammalians, toll-like receptors (TLR) are pattern recognition receptors, which are best known for their involvement in the induction of the innate immune system in response to pathogens, injury and disease. Also, recent evidence indicates that TLRs also play non-immune roles, which remain poorly defined. TLR9 is present in the central nervous system (CNS) where it is important for the appropriate development of sensory and motor function. The contribution of TLR9 in the CNS under pathological conditions, as for example Parkinson's disease (PD), has not been defined. In the present study, using mice genetically deleted for TLR9 (TLR9-/-) and their wild type (WT, C57BL/6) littermates we investigated the potential role of the TLR9 in the 6-hydroxydopamine (6-OHDA)-lesion model of PD. Female TLR9-/- and WT mice were used for the microinjection in the striatum of 6-OHDA (10 μg/mice) or a corresponding volume of saline (4 μl), unilaterally. While the number of rotations under apomorphine is related to the extent of dopamine depletion, mice were examined 21 days after the 6-OHDA lesion for turning behavior. Lesion intensity was analyzed by expression of Tyrosine hydroxylase (TH) immunohystochemistry. To establish a possible link between TLR9 function and neuroinflammation, glial cell activation was assessed by the extent of Iba1+ (microglia) and GFAP+ (astrocytes) cells within the striatum. TLR9−/− phenotype shows a hyper-responsive prepulse inhibition reaction and a decrease of exploratory and stereotype behavior, compared to WT controls. Accordingly, TLR9-/- mice showed increased number of apomorphine-induced rotations than WT group. In corroboration, we observed that TLR9-deficient mice displayed an increased density of surviving TH+ fibers in the dorsal striatum compared to WT mice (total density of TH-fibers: 43,75 ± 5,89, WT+6-OHDA; 65,50 ± 5,87, TLR9 deficient+6-OHDA), suggesting that TLR9-/- mice could be partially protected against 6-OHDA toxicity at the dopaminergic terminal. Moreover, after 21 days, TLR9-/- mice with 6-OHDA microinjection displays no changes in the density of Iba1+ activated microglial cells in comparison with WT group. However, there was a significant increase in the number of the astrocytes in the dorsal striatum. Taken together, we have demonstrated that TLR9-deficient mice are less vulnerable to 6-OHDA intoxication than C57BL/6 mice, suggesting that the TLR9 pathway is involved in experimental PD.

Disclosures: **A.C. Licursi De Alcantara:** None. **M. Bortolanza:** None. **F.M. Tristão:** None. **J.F.C. Pedrazzi:** None. **M.S. Pereira:** None. **R. Raisman-Vozari:** None. **T.M. Cunha:** None. **E. Del Bel:** None.

Poster

694. Parkinson's Disease

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: FAPESP

CNPq

CAPES

NAPNA

USP

Title: Cannabidiol attenuates sensorimotor gating disruption induced by amphetamine in mice

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Abstract: Introduction. Schizophrenia is a highly disabling disease, which would involve an imbalance in the dopaminergic neurotransmission and a glutamatergic hypofunction. The Delta9-tetrahydrocannabinol (Delta9-THC), the main psychotomimetic constituent of *Cannabis sativa*, induces psychotic reactions similar to schizophrenia symptoms, which are antagonized by cannabidiol (CBD). This component of *Cannabis* is devoid of psychotomimetic properties and appears to have pharmacological profile similar to antipsychotic drugs. Clinical studies have investigated the use of CBD as an alternative treatment for schizophrenia symptoms. However, few preclinical studies have been performed to support the clinical utility of CBD, and to reveal its mechanism of action. Sensorimotor gating deficit is characterized by disruption in the prepulse inhibition (PPI) response. Disruption in PPI is present in schizophrenia patients and can be reproduced in experimental conditions by psychotomimetic drugs, as amphetamine. Psychotomimetic-induced PPI disruption reproduces the positive symptoms of schizophrenia.

The ability of drugs to reverse PPI disruption is predictive of antipsychotic effects. **Objective**. The aim of this study was to investigate the ability of CBD to reverse the amphetamine disruptive effects in the PPI test. **Methods.** Male Swiss mice (25-35g) received an intraperitoneal (i.p.) injection of either vehicle or CBD (15, 30 or 60mg/kg) followed, 30 minutes after, of a second i.p. injection of saline (10ml/kg) or amphetamine (10mg/kg), and were submitted to the PPI test 30 minutes later. The PPI test consist of 64 trials irregularly divided into pulse (P, white noise, 105 dB), prepulse (PP; pure tone; 7kHz; 80, 85 or 90 dB), prepulse+pulse (PP+P) and no-stimuli with white background noise level of 64 dB - %PPI=[100-(PP+P/P)*100]. The percentage of PPI was analyzed with repeated measures with the treatment as the independent factor and the prepulse intensity as repeated measure. Duncan´s post hoc test ($p<0.05$) was used to specify differences. **Results.** The acute treatment with amphetamine promoted significant PPI disruption at all prepulse intensities analyzed. CBD blocked the disruptive effect of amphetamine in a dose (30 and 60mg/kg) and prepulse-dependent way (85 and 80 dB). CBD alone did not produce any PPI change. **Conclusion.** Our results demonstrate, for the first time, the ability of CBD to reverse amphetamine-disruptive effects in the PPI test. Our data corroborates the hypothesis of CBD antipsychotic profile.

Disclosures: J.F. Pedrazzi: None. **A.C.I. Pereira:** None. **F.V. Gomes:** None. **E.A. Del Bel:** None. **F.S. Guimarães:** None.

Poster

694. Parkinson's Disease

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CNPq

CAPES

NAPNA

USP

Title: Methylene blue effects in the psychotomimetic-elicited behaviors in mice

Authors: *A. DE CASTRO ISSY PEREIRA[1], J. F. C. PEDRAZZI[2], S. L. A. CALERO[4], E. DEL BEL[3];
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Abstract: Nitric oxide (NO) is an atypical neurotransmitter, which seems to be implicated in dopamine and glutamate related pathologies such as schizophrenia. The role of NO system had been demonstrated in some experimental schizophrenia-like symptoms. Following its synthesis at postsynaptic site NO may diffuse back to the presynaptic terminal and increase guanosine 3,5-monophosphate (cGMP) levels through activation of the heme group of the soluble guanilate cyclase (sGC), the major intracellular target of NO. Methylene blue (MB) was a lead compound to the development of the phenothiazine neuroleptic family. MB inhibits sGC/NOS activity with a low toxicity profile in preclinical and clinical studies. The aim of this study was to investigate the ability of lower doses of MB to modify behavioral effects induced by the psychomimetic drugs amphetamine (D1- and D2-like receptors agonist) or the non-competitive NMDA-receptor antagonist MK-80, as hyperlocomotion, stereotypies and prepulse inhibition (PPI) deficit. Male Swiss mice were pre-treated (intraperitoneally) with saline (10ml/kg) or MB at doses of 2 or 6mg/kg, followed (30 minutes after) by saline, amphetamine (10mg/kg) or MK-801 (0.5mg/kg). Stereotypies are behavioral responses known dependent of the increase of dopaminergic neurotransmission in the striatum dorsal. Therefore, the stereotypies induced by apomorphine (D2-like receptor agonist; 1 or 5mg/kg; subcutaneous) were evaluated in an independent experimental group. Repeated Measure-MANOVA was used for PPI analyses. Hyperlocomotion and stereotypies were analyzed by one-way ANOVA. Our results showed that acute treatment with either amphetamine or MK-801 induced a robust deficit in the PPI response, which was not modified by the MB pretreatment. Contrasting, MB at dose of 2mg/kg slightly reduced hyperlocomotion ($p<0.1$) induced by amphetamine treatment. MK-801 at dose tested marginally induced ataxia, which was not modified by MB pretreatment. Stereotypies were induced by both amphetamine and apomorphine treatment. However, MB pre-treatment reversed only the stereotypies induced by amphetamine. Our data suggested that the pharmacological effects of MB are probably related to its ability to inhibit sGC/NOS in the dorsal striatum. Also, MB effects appear to be mostly related to modulation of dopaminergic D1-like receptors effect, since the stereotypies induced by apomorphine were not modified. Complementary studies with D1 selective agonist may test this hypothesis. In conclusion, our data corroborate a potential therapeutic role of MB in pathologies related to striatal dysfunction.

Disclosures: A. de Castro Issy Pereira: None. **J.F.C. Pedrazzi:** None. **S.L.A. Calero:** None. **E. Del Bel:** None.

Poster

694. Parkinson's Disease

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: FAPESP

CNPq

CAPES

NAPNA

USP

Title: Prepulse inhibition response in rodents with striatal 6-hydroxydopamine lesion

Authors: *E. DEL BEL[1,2], A. ISSY[2], F. E. PADOVAN-NETO[2], M. LAZZARINI[3], M. BORTOLANZA[2];
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Abstract: Parkinson's disease (PD) is a progressive movement disorder that involves non-motor symptoms including cognitive dysfunction. The symptoms of PD can be ameliorated with dopamine replacement therapy through l-3 4-dihydroxyphenylalanine (L-DOPA). However, it might cause the development of abnormal involuntary movements (dyskinesia) and also psychotic symptoms. Psychosis, characterized by delusions and hallucinations, is one of the most serious L-DOPA side effects. The occurrence of a complex interaction between extrinsic/intrinsic, drug-related and disease related components has been argued regarding the psychotic symptoms of PD. Prepulse inhibition (PPI) is a cross-species robust measure of sensorimotor gating often disrupted in disorders either with basal ganglia dysfunction or psychotomimetic drugs. There are controversial results concerning PPI values in PD patients. Nevertheless, clinical studies are difficult to interpret because of confounding factors such as differences in disease severity, concomitant medications, and comorbidities. In the current study we conducted a series of PPI test in the 6-OHDA rodent model of PD, including also the investigation of other variables: i) animal species (mice or rat); ii) lesion localization (medial

prosencephalic bundle or striatum); and iii) lesion extension (unilateral or bilateral). Because several studies suggested that PD-associated psychosis results from interaction between disease-related factors and pharmacological replacement of dopamine we analyzed the effect of L-DOPA treatment (30 mg/kg, daily). We choose acute, subchronic (7 days) and chronic (14 days) treatment with L-DOPA. Unilateral striatal degeneration of tyrosine hydroxylase immunolabeled terminals in the 6-OHDA-lesioned rats, either complete (including nucleus accumbens) or partial did not significantly altered PPI response. Also, dorsal striatum unilateral or bilateral dopaminergic loss did not determine PPI changes. Similar findings were obtained with both Wistar rats and Swiss mice. L-DOPA treatment induced dyskinesia in 6-OHDA-lesioned rats at the dose tested. However, there were no changes in the PPI response induced by L-DOPA treatment. In conclusion, our results suggest a rather small/absent influence of dopamine depletion in the dorsal striatum in the PPI modulation. Also, it brings the proposition that the 6-OHDA animal model of PD do not easily reproduce the impairment of PPI response sometimes found in PD patients. Further investigation or other experimental conditions are required to better understand the effects of dopamine depletion in the sensorimotor gating.

Disclosures: E. Del Bel: None. **A. Issy:** None. **M. Lazzarini:** None. **F.E. Padovan-Neto:** None. **M. Bortolanza:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Title: Microglial TNF-α mediates enhancement of dopaminergic degeneration by brain angiotensin

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Abstract: *In vitro* and *in vivo* models of Parkinson's disease were used to investigate whether TNF-α plays a major role in the enhancement of the microglial response and dopaminergic degeneration induced by brain angiotensin hyperactivity. Treatment of primary mesencephalic cultures with low doses of the neurotoxin MPP^+ induced a significant loss of dopaminergic neurons, which was enhanced by co-treatment with angiotensin II and inhibited by TNF-α inhibitors. Treatment of primary cultures with angiotensin induced a marked increase in levels of TNF-α, which was inhibited by treatment with angiotensin type-1-receptor antagonists, NADPH-oxidase inhibitors and NFK-β inhibitors. However, TNF-α levels were not significantly affected by treatment with angiotensin in the absence of microglia. The microglial origin of the angiotensin-induced increase in TNF-α levels was confirmed using dopaminergic (MES 23.5) and microglial (N9) cell lines. Inhibition of the microglial Rho-kinase activity also blocked the AII-induced increase in TNF-α levels. Treatment of the dopaminergic cell line with TNF-α revealed that NFK-β activation mediate the deleterious effect of microglial TNF-α on dopaminergic neurons. Treatment of mice with MPTP also induced significant increases in striatal and nigral TNF-α levels, which were inhibited by angiotensin type-1-receptor antagonists or NFK-β inhibitors. The present results show that microglial TNF-α plays a major role in angiotensin-induced dopaminergic cell death and that the microglial release of TNF-α is mediated by activation of angiotensin type-1 receptors, NADPH-oxidase, Rho-kinase and NFK-β.

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Poster

695. Mechanisms of Cell Death and Dysfunction

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Topic: C.03. Parkinson's Disease

Support: ANR-10-IAIHU-06

ANR-2010-BLAN-1418-01

Title: Defining the role of chemokines as modulators of pathological neuro-glia-immune interactions during neurodegeneration in Parkinson's disease

Authors: *R. V. PARILLAUD[1], G. LORNET[1,2], Y. MONNET[1], C. COMBADIÈRE[3], E. C. HIRSCH[1], S. HUNOT[1], C. S. LOBSIGER[1];
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Abstract: Neuroinflammatory processes and non-cell autonomous mechanisms can contribute to neuronal demise in diverse neurodegenerative disorders. In Parkinson's disease (PD), activated glia and infiltrating immune cells are present around degenerating dopaminergic (DA) neurons and studies in mice revealed that suppression of microglial effectors or deletion of infiltrating lymphocytes can reduce DA neurodegeneration. However, little is known about the signaling molecules driving these pathological neuro-glia-immune interactions in PD. The basic concept suggests that affected DA neurons produce stress-signals that activate neighbouring glia, which in turn produce signals to attract peripheral immune cells. As chemokines represent excellent candidates for such cell-cell signaling, our aim was to assess in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) mouse model of PD, if chemokines act as neuronal and/or glial derived signals to modulate neuroinflammation and/or DA neurodegeneration. We applied a 2-step laser-microdissection approach using RNA pre-amplification and TaqMan qPCR arrays. First, we screened the full chemokine family (34 ligands, 20 receptors) at 3 timepoints during MPTP-induced DA neurodegeneration in the affected Substantia nigra pars compacta (SNpc). Second, focusing on early induced chemokines, we assessed their cellular origin in pools of neurons and surrounding glia, and identified a set of promising candidates (CCL2/3/4/5/7/12, CXCL10/14/16) with corresponding receptors early induced in the SNpc. Immunohistochemical confirmation in MPTP mice revealed early induction of astrocytic CCL2/7 and microglial CCL12 for the CCL2/7/12-CCR2 axis and CXCL16 in microglial subpopulations for the CXCL16-CXCR6 axis. The former axis suggests prescence of infiltrating inflammatory monocytes, while the latter could be implicated in the deleterious infiltration of lymphocytes. With respect to the strongly debated infiltration of monocytes, and to avoid artefactual effects from irradiation/BMT, we use BAC-CCR2-GFP reporter mice in combination with MPTP and CCR2-deletion or CCL2-overexpression to assess the prescence of infiltrating monocytes and their capacity to modulate DA neurodegeneration. With respect to the CXCL16-CXCR6 axis, we use CXCR6-GFP mice to trace infiltrating lymphocyte subpopulations and after systemic gene deletion or transfer of CXCR6-deleted spleenocytes into Rag recipient MPTP mice, assess their contribution to ongoing DA neurodegeneration. Our results shed light on the complex cellular expression and disease modifying capacity of the chemokine network during DA neurodegeneration.

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Poster

695. Mechanisms of Cell Death and Dysfunction

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HD02528

Title: Effects of discontinuing a high-fat diet on 6-hydroxydopamine-induced nigrostriatal dopamine depletion in rats

Authors: *D. MA, J. M. SHULER, K. D. RAIDER, R. S. ROGERS, J. L. WHEATLEY, P. C. GEIGER, J. A. STANFORD;
Univ. of Kansas Med. Ctr., KANSAS CITY, KS

Abstract: Regular consumption of a diet high in fat has been shown to increase the risk for developing age-related neurodegenerative diseases such as Parkinson's disease (PD). Recent studies from our lab and others demonstrate that a high-fat diet increases dopamine (DA) depletion in the MPTP mouse and 6-hydroxydopamine (6-OHDA) rat models of PD. These results suggest that switching to a low fat diet should decrease neuronal vulnerability under these conditions. Despite this prediction, little is known about the long-term effects of diet-induced obesity on neuronal vulnerability to PD. Recent studies suggest that a high-fat diet may produce effects that persist even after a switch to a healthier, low-fat diet. The goal of this study was to test the hypothesis that the increased vulnerability to 6-OHDA-induced nigrostriatal DA depletion exhibited by rats fed a high-fat diet persists after switching to a low fat diet. We fed rats diet comprised of 60% calories from fat for 12 weeks. We then switched half of these rats to a regular chow diet for 12 weeks (HF-chow rats) while the other half continued with the high-fat diet (HF rats). A chow-fed group was included as a control. We then infused 6-OHDA into the nigrostriatal pathway and allowed the rats to recover for 2 weeks. Rats underwent amphetamine-induced rotation testing and an intraperitoneal glucose tolerance test (IPGTT) prior to tissue harvest. Whole tissue levels of DA were measured using HPLC-EC in lesioned and unlesioned striata. There was no difference in amphetamine-induced rotation between the three groups. HF rats had significantly higher fasting insulin levels and higher HOMA-IR, as well as higher glucose levels during the IPGTT, indicating insulin resistance in this group. These variables were significantly lower in HF-chow rats, indicating that high-fat diet-induced insulin resistance was reversed by switching to regular chow. We did not observe significant differences in DA

depletion between the three groups. However, lower DA content was measured in both the lesioned and unlesioned striata from HF and HF-chow rats compared to controls. The implications of these findings on potentially persisting effects of a high-fat diet on vulnerability to neurodegeneration following a switch to a low-fat diet are unclear, but they do suggest differences between peripheral and central outcomes.

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Poster

695. Mechanisms of Cell Death and Dysfunction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 695.04/D72

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS65167

NIH Grant ES10586

NIH Grant ES078327

Title: Involvement of mitochondrial dysfunction and autophagic alterations in tumor necrosis factor like weak inhibitor of apoptosis (tweak) induced dopaminergic neuronal injury

Authors: S. KANURI, H. JIN, V. ANANTHARAM, *A. KANTHASAMY, A. KANTHASAMY;
Biomed. Sci., Iowa State Univ., Ames, IA

Abstract: Neuroinflammation, oxidative damage, mitochondrial dysfunction, and impaired clearance of aggregate prone proteins are implicated in Parkinson's Disease (PD) pathogenesis; however, the potential cross talk between exaggerated inflammatory response and impaired protein degradation machinery is poorly understood. Recently, using both *in vitro* and *in vivo* models of PD we demonstrated that Tumor Necrosis Factor like Weak Inducer of Apoptosis (TWEAK) induced dopaminergic cell death may be linked to microglial activation. Furthermore, a strong induction of TWEAK was evidenced in MPP+ & MnCl2 treated dopaminergic neuronal cells. Likewise, a significant increase in TWEAK levels were evidenced in the serum of PD patients as compared to age matched controls. Therefore, in the present study we sought to investigate the cell signaling events that underlie the increased susceptibility of DA gic neurons

to TWEAK induced apoptotic cell death using an *in vitro* dopaminergic cell culture model, N27 cells. Herein, we show that N27 cells express both TWEAK and its receptor Fn14. After this initial finding, we found that TWEAK-elicited dose dependent apoptotic cell death in N27 cells. Exposure of N27 cells to TWEAK evoked dissipation of mitochondrial membrane potential (MMP), suppression of GSH levels and caspase 3 activation and concomitant up regulation of Phospho-Tau and Phospho-NF-kB P65 levels. Conversely, TWEAK-induced down regulation of Phospho-AKT and Phospho-p44/42 MAPK (ERK 1/2) levels were accompanied by decreased P-GSK3Beta and LC3 levels in dopaminergic neuronal cells. Intriguingly, quercetin, a bioflavanoid afforded protection against TWEAK induced dopaminergic cell death. A dose dependent study performed in N27 cells revealed that, quercetin afforded protection against TWEAK induced cytotoxicity by attenuating the drug-induced dissipation of MMP at lower doses; although, at higher doses toxic effects were evidenced. In a similar fashion SN50, a peptide inhibitor of NF-kB also abrogated TWEAK induced apoptotic cell death in N27 cells. Together, these data suggest that TWEAK exerts deleterious effects on dopaminergic neuronal survival presumably via sequential deregulation of mitochondrial function and enhanced trans-activation of NF-kB as well as compromised protein degradation machinery.

Disclosures: S. Kanuri: None. **H. Jin:** None. **V. Anantharam:** None. **A. Kanthasamy:** None. **A. Kanthasamy:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Program#/Poster#: 695.05/E1

Topic: C.03. Parkinson's Disease

Support: Seoul National University Bundang Hospital (02-2005-027)

Research Center Program of the IBS (Institute for Basic Science) in South Korea

Title: Mapping of cellular iron in the *in vitro* model of Parkinson's disease with high-resolution hyperspectral imaging

Authors: *C. HEO[1], E. OH[3], H. SHIM[2], K. HAN[2], H. RYU[2], J.-M. KIM[3], M. SUH[2];
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Kwan Univ., Suwon City, Korea, Republic of; [3]Dept. of Neurology, Seoul Natl. Univ. Bundang Hosp., Seongnam City, Korea, Republic of

Abstract: Dopaminergic neurons are located in a subarea of the substantia nigra (SN) and the pars compacta. Progressive loss of these neurons is a pathological hallmark of Parkinson's disease (PD), together with abnormally high deposition of iron in this area. The elevated iron level in the SN of PD has been demonstrated by autopsy, and with 7-Tesla magnetic resonance imaging. To elucidate the potential role of iron in developing PD, it is important to quantify the cellular iron deposit and recognize iron distribution pattern. Direct visualization of cellular iron with live cell-imaging techniques, however, has not been successful. The aim of this study is to visualize and quantify the distribution of cellular iron using an intrinsic iron hyperspectral fluorescence signal without any cell lysis and chemical markers. The 1-methyl-4-phenylpyridinium (MPP+)-induced SHSY5Y cells exposed to iron with ferric ammonium citrate (FAC, 100 μM) for 1-hour and 6-hour duration, respectively. The hyperspectral fluorescence signal of iron was examined using a high-resolution (optical resolution near 100 nm) dark-field optical microscope system with signal absorption for the visible/near infrared spectral range. The 6-hour group showed heavy cellular iron deposition compared with the 1-hour group. The cellular iron was dispersed in a small particulate form, whereas the extracellular iron was rather largely aggregated. In addition, iron particles were found to be concentrated on the cell membrane and the edge of shrunken cells. The iron was readily accumulated in MPP+-induced cells, which is consistent with previous studies demonstrating elevated iron levels in the SN. Thus, this study suggests that the direct iron imaging can be applied to investigate the physiological role of iron in PD. Furthermore, this technique may be applicable to various neurological disorders involving deposition of metals, such as copper, manganese, or zinc.

Disclosures: C. Heo: None. **H. Shim:** None. **K. Han:** None. **H. Ryu:** None. **M. Suh:** None. **E. Oh:** None. **J. Kim:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Program#/Poster#: 695.06/E2

Topic: C.03. Parkinson's Disease

Support: NIH/NCMHD P20MD003352

Title: Inhibition of HRD1 ligase activity by alpha synuclein

Authors: *J. R. ZYSK[1], T. JOSEPH[1], A. COLLIER[2], A. ELLIS[3];
[1]Chem., [2]Biol., [3]Biochem., Lincoln Univ., Lincoln University, PA

Abstract: Parkinson's Disease (PD) is associated with cellular stress induced by the unfolded protein response (UPR). It has been suggested that accumulation of the PD-related protein alpha synuclein (αS) induces UPR in the endoplasmic reticulum (ER) where degradation of misfolded proteins normally occurs. This ER-associated degradation (ERAD) involves polyubiquitination of misfolded proteins through the ubiquitin-proteosome system (UPS). This pathway involves E3 ligases, such as parkin, a protein linked to juvenile onset PD and which targets the parkin-associated endothelin receptor-like receptor (Pael-R). In addition, parkin over-expression has been found to reverse αS-induced cellular toxicity. Another ERAD-associated E3 ligase recently suggested to play a role in PD (also using Pael-R as a substrate) is HRD1. Over-expression of HRD1 in HEK cells was found to decrease Pael-R-induced cell death (Omura, et al., 2013). Moreover, the antiepileptic Zonisamide which upregulates HRD1, was found to suppresses neuronal cell death induced by 6-hydroxydopamine, a laboratory model for PD (Omura, et al., 2012). Although some evidence suggests that αS is an E3 (parkin) substrate, we propose that it affects E3 ligase activity in a different manner. An ELISA-based ligase assay (LifeSensors, Malvern, PA), was used to measure the effect of αS on HRD1 ligase activity. The E3 ligase CARP2, which is not associated with PD, was used as a positive control. Our results indicate that αS exhibits an inhibitory effect on HRD1 at molar ratios ranging from 6:1 to 3:1 (60 or 30 nM αS to10 nM HRD1) at an incubation period of 40 minutes at room temperature. The effect was most pronounced (>50% inhibition) when the time-sensitive reagents were used within three days of receipt. When tested three weeks later, the inhibition had dropped to 26% at 60 nM αS. No consistent effect was observed at incubation times under 40 minutes. A small (~3%) inhibitory effect by αS on CARP1 was observed at 40 minutes. While these data suggest a role for αS in modulating E3 ligase function, the study was limited in scope and performed in an end point assay rather than one using real time kinetics. However, these data suggest a disease-relevant mechanism for PD.

Disclosures: J.R. Zysk: None. **T. Joseph:** None. **A. Collier:** None. **A. Ellis:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Title: Alpha-Synuclein misfolding versus aggregation in Parkinson's disease: Critical assessment and modeling

Authors: *V. VASQUEZ, R. BERROCAL, S. RAO KRS, J. RAO;
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Abstract: α-Synuclein, an abundant and conserved presynaptic brain protein, is implicated as a critical factor in Parkinson's disease (PD). The aggregation of the α-synuclein is believed to be a critical event in the disease process. α-Synuclein is characterized by a remarkable conformational plasticity, adopting different conformations depending on the environment. Therefore, it is classified as an 'intrinsically disordered protein'. Recently, a debate has begun over how α-synuclein behaves in the cell: is it an intrinsically disordered protein or a stable tetramer with a low propensity for aggregation? In our critical analysis, we discussed about the major questions: i) why α-synuclein conformational behavior doesn't fit into the normal secondary structural characteristics of proteins?, ii) what amino acids are responsible for its misfolded nature leading to aggregation?, and iii) How metals will influence misfolding and aggregation? To analyze the above critical questions, we developed bioinformatics models related to secondary and tertiary conformations, Ramachandran plot, free energy change, intrinsic disordered prediction, solvent accessibility, and FoldIndex pattern. To the best of our knowledge, this is a novel critical assessment to understand the misfolding biology of Synuclein and its relevance to Parkinson disease.

Disclosures: **V. Vasquez:** None. **R. Berrocal:** None. **S. Rao KRS:** None. **J. Rao:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS059869

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Title: α-Synuclein activates apoptosis signal regulating kinase 1 in models of Parkinson's disease

Authors: E. PARK, K.-W. LEE, J.-M. WOO, J.-Y. IM, E. JUNN, *M. M. MOURADIAN; Neurol., Rutgers-Robert Wood Johnson Med. Sch., PISCATAWAY, NJ

Abstract: Evidence from human genetic studies, animal models and cell biologic experiments indicate that over-expression of α-synuclein is deleterious to neurons. Various mechanisms have been identified to contribute to this increased neuronal vulnerability including oxidative stress. Apoptosis Signal-Regulating Kinase 1 (ASK1) belongs to the MAP3 kinase family that is activated by various stimuli including oxidative stress and relays those signals to JNK and p38 kinase leading to apoptosis. In Parkinson's disease (PD), ASK1 has been shown to be activated and co-localized with α-synuclein in Lewy bodies of nigral neurons. We had previously demonstrated that challenging dopaminergic neuroblastoma SH-SY5Y cells with MPP+ as well as systemic exposure of mice to MPTP result in phosphorylation/activation of ASK1. In the present study, we sought to determine if α-synuclein over-expression regulates ASK1 activity as well. PC12 cells were engineered to express α-synuclein using the Tet-off system, differentiated with nerve growth factor for 12 to 48 hr, and lysates were assessed by Western blots for ASK1 and α-synuclein expression. Induction of α-synuclein expression following removal of doxycycline from the culture medium was associated with time-dependent increased phospho-ASK1 expression but no appreciable change in total ASK1 levels. This result indicates that α-synuclein over-expression leads to ASK1 activation in a simple cellular model. Next, we explored the state of ASK1 activation in the cerebral cortex of 3-month old transgenic mice over-expressing human α-synuclein under the control of the pan-neuronal Thy-1 promoter. Levels of phosphorylated ASK1 on Western blots were ~3 fold higher in α-synuclein transgenic mice compared with wild-type animals. This was confirmed by immunohistochemistry of cortical tissue sections with phospho-ASK1 antibody. Total ASK1 expression was no different between the two mouse groups. These results collectively indicate that α-synuclein over-expression leads to ASK1 activation and suggest that one of the mechanisms for α-synuclein induced toxicity is mediated through ASK1. Thus, inhibiting this apoptotic kinase has the potential to exert neuroprotective effects in α-synucleinopathies such as PD. Supported by NIH grants NS059869 and NS053517

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Poster

695. Mechanisms of Cell Death and Dysfunction

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Alfried Krupp prize

Title: Cav1.3 L-type calcium channels control age-dependent D2-autoreceptor responses via NCS-1 in Substantia nigra dopamine neurons

Authors: E. DRAGICEVIC[1], C. POETSCHKE[1], J. DUDA[1], F. SCHLAUDRAFF[1], S. LAMMEL[2], J. SCHIEMANN[3], M. FAULER[1], A. HETZEL[4], M. WATANABE[5], R. LUJAN[6], R. C. MALENKA[2], J. STRIESSNIG[7], *B. LISS[1];
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Abstract: Dopamine neurons within the Substantia nigra (SN DA), are important for voluntary movement control and are particularly prone to degeneration in Parkinson's disease (PD). Their loss causes the major motor-symptoms of PD, but the cause for their high vulnerability to degeneration, compared to neighboring, more resistant ventral tegmental area (VTA) DA neurons, is still unclear. Accordingly, no curative therapy for PD is available. Existing

symptomatic therapies aim to compensate the progressive loss of dopamine by administering its precursor L-DOPA and/or dopamine D2-receptor agonists. D2-autoreceptors (D2-ARs), which control firing-rates and dopamine release of SN DA neurons in a negative feedback-loop via activation of G-protein coupled potassium channels (GIRK2), display pronounced desensitization in PARK PD mouse models. Blood-brain-barrier permissive L type Ca2+ channel (LTCC) blockers protect SN DA neurons in PD and its mouse models, and are already in clinical trials. However, their protective mechanism, as well as the physiological functions of LTCCs in SN DA neurons, remain unclear. Thus both, D2 ARs and Cav1.3 are involved in PD-pathology and its therapy, but their interplay is unclear. We analyzed remaining human SN DA neurons from PD patients and controls, and detected elevated mRNA-levels of D2-AR and GIRK2 in PD. By electrophysiological analysis of postnatal juvenile and adult mouse SN DA neurons in *in vitro* brain-slices, we observed that D2-AR desensitization is reduced with postnatal maturation. Furthermore, a transient high-dopamine state *in vivo*, caused by one injection of either L-DOPA or cocaine, induced adult-like, non-desensitizing D2-AR-responses, selectively in juvenile SN DA but not VTA DA neurons. With pharmacological and genetic tools, we identified that the expression of this reduced D2-AR-desensitization phenotype required Cav1.3 activity, internal free Ca2+, and the interaction of the neuronal calcium sensor NCS-1 with D2-ARs. Thus, we identified a first physiological function of Cav1.3 LTCCs in SN DA neurons, for homeostatic modulation of their D2-AR responses. LTCC-activity however, was not important for SN DA pacemaker-activity. We also detected elevated mRNA-levels of NCS-1 (but not of Cav1.2 or Cav1.3) after cocaine in mice - and in human SN DA neurons in PD. Thus, our findings provide a novel link in SN DA neurons between Cav1.3 and D2-AR activity, controlled by NCS-1, and indicate that this adaptive signaling network (Cav1.3/NCS-1/D2-AR/GIRK2) is also active in human SN DA neurons, and contributes to PD-pathology. It provides a novel target for tuning SN DA activity, and their vulnerability to degeneration.

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Poster

695. Mechanisms of Cell Death and Dysfunction

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 695.10/E6

Topic: C.03. Parkinson’s Disease

Title: Environmental toxicant-induced decrease in GM1 ganglioside expression in dopamine neurons: Potential mechanism contributing to development of Parkinson's disease

Authors: *T. MORRISON[1], D. W. ANDERSON[1], J. CAI[2], L. IACOVITTI[2], J. S. SCHNEIDER[1];
[1]Pathology, Anat. & Cell Biol., [2]Neurosci., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Parkinson's disease (PD) is a complex neurodegenerative disorder, the causes of which are still unknown. Epidemiological studies suggest that exposure to organic pollutants including pesticides (and related subcategories of herbicides, insecticides, and fungicides) is associated with the development of PD. Yet, the cellular and molecular mechanisms that may lead from environmental exposures to dopamine (DA) neuron degeneration and development of PD are not known. GM1 ganglioside plays important roles in cell functioning and survival and appears to be decreased in DA neurons in PD. A decrease in GM1 expression may play a role in the response of DA neurons to various insults, including exposures to pesticides and other toxicants. The present studies investigated the extent to which pesticide exposures may affect GM1 expression in DA neurons and contribute to their response to this injury. Mouse E14.5 hTH-GFP SN cells were dissected and plated on poly-D-lysine coated chamber slides. Two days later, some wells were treated with maneb (MB, 30 μM) for 24 hours before fixation and processing for GM1 immunohistochemistry. MB exposure did not damage or decrease the number of TH+ cells but decreased GM1 expression on TH+ neurons. In a second study, mouse E14.5 hTH-GFP SN cells were dissected and plated as described above. Two days after dissection, half of the wells were treated with 200 μM GM1 for 2 hours and then were treated with either MB or MPP+ with or without GM1 for 24 hrs before fixation. Counts of hTH-GFP+ cells showed that both MB and MPP+-damaged cells were responsive to GM1 treatment, suggesting a potentially common response of these cells to these toxins that can be negated by GM1 replacement. To examine whether low level pesticide exposures *in vivo* can also decrease GM1 expression in DA neurons, adult male C57Bl6 mice were injected with MB (10 mg/kg) + paraquat (PQ, 30 mg/kg) twice per wk. (4 days apart) for 2 wks and euthanized 1 wk after the last pesticide exposure. Striatal DA levels were normal but decreased GM1 expression was observed in TH+ neurons in the substantia nigra. These data support the hypothesis that pesticide exposure can decrease GM1 expression in otherwise healthy appearing DA neurons and we suggest that this may adversely affect the way these cells respond to other subsequent stressors.

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Poster

695. Mechanisms of Cell Death and Dysfunction

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 695.11/E7

Topic: C.03. Parkinson's Disease

Title: Correlative light and electron microscopy to elucidate the function of genes implicated in Parkinson´s disease

Authors: *F. KLEINE BORGMANN[1,2], A. SKUPIN[1], M. ELLISMAN[2];
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Abstract: Parkinson's disease (PD) is a common neurodegenerative movement disorder that is characterized by the selective loss of dopaminergic neurons (DN) in the substantia nigra. The causes are largely unknown and no cure is known; treatment focuses on alleviating the symptoms by mimicking the function of the DN by either administration of L-dopa or deep brain stimulation. An important step forward in understanding PD is the identification of key genes involved and to learn how these genes and the encoded proteins function and malfunction in case of the disease. Several genes have been identified as being implicated in disease development as their mutations are frequently found in inherited forms of PD. For some of these genes certain functions are known already from different contexts; many of them are involved in mitochondrial turnover and protein degradation pathways. However why their malfunctions lead to this highly distinct condition where only a small set of cells is affected is not understood. We aim to localize the candidate proteins on a cellular and subcellular level in different cell types of the brain, characterize their dynamics and shed light on disease progression. For this purpose, we perform correlated light and electron microscopy using mice as a model organism. We generate high-resolution 3D datasets of different areas of the brain, denoting the exact location of several PD-implicated proteins and their function in energy metabolism. The data obtained by this project will lead to a systematic understanding of the molecular dynamics of PD and help in the development of personalized medical approaches for different forms of PD.

Disclosures: F. Kleine Borgmann: None. **A. Skupin:** None. **M. Ellisman:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Program#/Poster#: 695.12/E8

Topic: C.03. Parkinson's Disease

Support: UTPA Faculty Research Council

Title: Early neuronal consequences of rotenone toxicity in Lymnaea stagnalis and possible relvance to Parkinson's Disease

Authors: J. CARRIZALES, H. CANTU, M. E. CANTU GUTIERREZ, *D. T. PLAS;
Biol., The Univ. of Texas-Pan American, Edinburg, TX

Abstract: Rotenone, a toxin that occurs naturally in some plants, has been used in several animal species to model aspects of Parkinson 's Disease. The toxin is widely believed to target Protein Complex I in the mitochondrial Electron Transport Chain, and mitochondrial dysfunction is a key feature of both inherited and sporadic cases of human Parkinson's Disease. In the freshwater gastropod, *Lymnaea stagnalis*, acute rotenone exposure reduces locomotion and feeding behavior and others have reported that perturbations of the brain's dopaminergic system follow. At the cellular level, we find that rotenone exerts strong effects on the morphology and dynamics of mitochondria in the neurons of the Central Nervous System. Neural mitochondria were found to exhibit significant reduction in volume along with other morphological changes indicating an altered dynamics. However, we find that rotenone causes only moderate reduction in Mitochondrial Membrane Potential. One possible explanation of this finding is that damaged mitochondria are actively removed by an increased autophagy activity. Consistent with this possibility, our Western blot analysis finds increased expression of the autophagic marker, Atg8 in treated animals. Given Rotenone's effect on the Electron Transport Chain, it might also be expected that Reactive Oxygen Species (ROS) generated would lead to protein damage which then might be expected to activate the cellular protein clearance machinery. The Ubiquitin Proteasome System (UPS) is the cell's ordinary agent of protein clearance, using and elaborate ubiquitin-tagging system to target proteins for degradation. We measure changes in ROS production, changes in protein carbonyl concentration in total brain protein, and levels of protein ubiquitination. Our work finds significant increases in both levels of protein damage and in the overall ubiquitination levels of proteins in neurons exposed to Rotenone. These results support the continued use of the *in vivo Lymnaea stagnalis*-rotenone system as a tool to study pathological cellular processes that also occur in Parkinson's Disease.

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Poster

695. Mechanisms of Cell Death and Dysfunction

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Topic: C.03. Parkinson's Disease

Support: Korea NFR Grant MRC 2008-0062275

Title: Enhancement of MPTP-induced dopaminergic neurotoxicity in IL-32β-overexpressed mice

Authors: *D.-Y. CHOI[1], Y.-Y. JUNG[2], J. HONG[2];
[1]Pharm., Yengnam Univ., Gyeongsan, Korea, Republic of; [2]Chungbuk Natl. Univ., Cheongju, Korea, Republic of

Abstract: IL-32 is a newly-found proinflammatory cytokine produced mainly by T cells, natural killer cells and epithelial cells. The cytokine plays a cardinal role in regulating innate and adaptive immune responses via activating p38 MAPK and NF-kappaB signaling pathways. Furthermore, IL-32 has been implicated in cancers, autoimmune diseases, inflammatory diseases and infectious diseases. However, no studies have described its role in dopaminergic neurodegeneration in Parkinson's disease models. In this study, we attempted to reveal the effects of IL-32 on dopaminergic neurotoxicity induced by MPTP via employing IL-32 transgenic mice. Immunohistochemical analysis disclosed that MPTP injection (15 mg/kg, 4 times) caused significant loss of dopaminergic neurons in the substantia nigra of non-transgenic and transgenic mice. Importantly, the loss was larger in IL-32 transgenic mice. In consistent, deletion of tyrosine hydroxylase-positive fiber density and depletion of dopamine in the striatum were more evident in transgenic mice. Behavioral tests showed that locomotor activity was more severely impaired in IL-32-overexpressed mice. Additionally, we found that glial activation was more profound in transgenic than non-transgenic mice. Overexpression of IL-32 significantly raised MPTP-induced expression of proinflammatory proteins including iNOS and COX-2. Finally, IL-32 overexpression exaggerated MPTP-mediated activation of p38 MAPK, JNK and ERK pathways. These results suggest that IL-32 might exacerbate MPTP-induced dopaminergic neurotoxicity through enhancing neuroinflammatory responses. Further study is necessary to elucidate its role in neurodegenerative diseases in humans.

Disclosures: **D. Choi:** None. **Y. Jung:** None. **J. Hong:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Program#/Poster#: 695.14/E10

Topic: C.03. Parkinson's Disease

Title: Modeling protein interactions related to Parkinson's Disease in silica

Authors: A. D. LEE, *B. BEHROUZ;
NeuroInitiative, Inc, Jacksonville Beach, FL

Abstract: Parkinson's disease is a progressive neurodegenerative disorder characterized by abnormalities and degeneration in several neuronal systems but most extensively in the nigrostriatal dopaminergic neurons. The cause and exact mechanisms remain unknown, however, several genes have been discovered that point to possible pathways responsible for progressive degeneration of these neurons. The details of these heavily interconnected pathways are difficult to unravel using traditional methods. In silica computer simulation can elucidate mechanisms that may otherwise go unnoticed using basic laboratory research. Computer modeling also allows researchers the ability to manipulate proteins within the system to mimic mutations that contribute to the disease or drugs that affect individual targets. The in silica modeling can reduce the occurrence of negative data and aide in development of hypotheses that lead to therapeutic targets.

Disclosures: **A.D. Lee:** None. **B. Behrouz:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Program#/Poster#: 695.15/E11

Topic: C.03. Parkinson's Disease

Support: NINDS Grant #NS065338

Title: The effects of acute neurotoxicant exposure on parkin expression and proteasome activity in brain regions containing differentially susceptible dopaminergic neurons

Authors: *T. LANSDELL[1], J. L. GOUDREAU[2], K. J. LOOKINGLAND[3];
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Abstract: Parkinson disease (PD) is a neurodegenerative disorder in which motor symptoms result from the loss of nigrostriatal dopamine (NSDA) neurons, while those in the mediobasal hypothalamus are spared. The pattern of dopamine (DA) neuron susceptibility to neurodegeneration can be recapitulated with neurotoxicant exposure, including acute and chronic treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone. Expression of parkin mRNA and protein increases in the medial basal hypothalamus following MPTP exposure, a change that is temporally linked to the phenotypic and functional recovery of tuberoinfundibular dopamine (TIDA) neurons in the mediobasal hypothalamus. In contrast, there is no increase in parkin mRNA or protein in the ventral midbrain following MPTP exposure and these neurons do not recover function or phenotype. Parkin is an E3 ubiquitin ligase with an N-terminal ubiquitin like domain that can directly modulate the activity of the proteasome.To assess the direct role of differentially expressed parkin on proteostasis within regions containing different DA neuron populations, proteasome activity was measured in wild type mice after treatment with MPTP using a fluorescent substrate for chymotryptic acitivity of the proteasome (Suc-LLVY-AMC). Four hours after exposure to MPTP, proteasome activity decreased in the striatum, nucleus accumbens, substantia nigra and arcuate nucleus, but remained unchanged in the median eminence. Compared to vehicle treated animals, levels of the catalytic β5, subunit were significantly decreased in the striatum and increased in the median eminence of MPTP treated animals. Twenty-four hours following MPTP exposure, parkin levels decreased in the striatum and proteasome activity remained diminished despite increased levels of β5 catalytic subunit. In contrast, proteasome activity in the arcuate nucleus recovered, which corresponded with increased levels of parkin, but levels of β5 were unaltered. Basal proteasome activity in the striatum and median eminence of parkin deficient mice was significantly decreased compared to wild type mice. These results are consistent with the conclusion that parkin plays a direct role in the recovery of proteasome activity after MPTP intoxication in neurons that are neurodegeneration resistant.

Disclosures: T. Lansdell: None. **J.L. Goudreau:** None. **K.J. Lookingland:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 695.16/E12

Topic: C.03. Parkinson's Disease

Support: KAKENHI 23890130

Title: Involvement of HDAC10 in MPP+-treated neuronal cell death and in brains with Parkinson's disease

Authors: *Y. NAGANO, T. KURASHIGE, E. DOHI, T. SHISHIDO, T. TAKAHASHI, H. MARUYAMA, M. MATSUMOTO;
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Abstract: Parkinson's disease (PD) is characterized by the progressive loss of nigrostriatal dopamine neurons and the formation of pathological hallmark, Lewy body. Impairment of autophagy has been shown to result in protein aggregation, leading to Lewy body formation. Recent paper showed that histone deacetylase (HDAC) 10 promoted autophagy-mediated cell survival and protected cancer cells from cytotoxic agents. However, the function of HDAC10 in neurodegenerative diseases remains unclear. In the present study, pathological studies showed that HDAC10 localized in the halo part of Lewy body in PD brains. Immunofluorescence studies showed that HDAC10 was clearly colocalized with α-synuclein which is the main component of Lewy body. We also checked that the protein expression levels of HDAC10 in PD patient's brain and control brains by western blotting. The result suggested that the expression levels of HDAC10 in PD brains are significantly higher than those of control brains. Furthermore, we found that knockdown of HDAC10 using siRNA enhanced neuronal cell death compared to control cells after MPP+ treatment. Interestingly, the number of cells containing protein aggregates were increased in HDAC10 knockdown cells compared to normal control cells, suggesting that HDAC10 could be involved in the clearance of protein aggregates via autophagy. Taken together, HDAC10 would play an important role in Lewy body formation and neuronal cell survival in PD brains by mediating autophagy pathway.

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Poster

695. Mechanisms of Cell Death and Dysfunction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 695.17/E13

Topic: C.03. Parkinson's Disease

Title: Role of E2 ubiquitin conjugating enzymes in Parkin-dependent mitochondrial quality control

Authors: *F. C. FIESEL, E. L. MOUSSAUD-LAMODIÈRE, M. ANDO, T. CAULFIELD, W. SPRINGER;
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Abstract: Loss-of-function mutations in the genes encoding PINK1 and Parkin are the most common causes of recessive Parkinson's disease (PD). Both together mediate the selective degradation of mitochondrial proteins and whole organelles via the proteasome and the autophagy/lysosome pathway (mitophagy). The mitochondrial kinase PINK1 activates and recruits the E3 ubiquitin (Ub) ligase Parkin to deenergized mitochondria. However, Parkin's cognate E2 co-enzymes in this Ub-dependent pathway have not been investigated so far. Using RNAi combined with High Content Imaging, we analyzed the effects of E2 knockdown on Parkin translocation in HeLa cells. Secondary biochemical assays were used to determine the effects on Parkin's activation and enzymatic functions. Our study uncovered redundant, cooperative or antagonistic effects of distinct E2 enzymes in the regulation of Parkin, reflecting their different functions along the course of mitophagy. Our results suggest a putative role of distinct E2 enzymes in PD pathogenesis.

Disclosures: F.C. Fiesel: None. **E.L. Moussaud-Lamodière:** None. **M. Ando:** None. **T. Caulfield:** None. **W. Springer:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

Location: Halls A-C

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Program#/Poster#: 695.18/E14

Topic: C.03. Parkinson's Disease

Support: Neuro Canada

Krembil Foundation

Parkinson Society Canada

Title: Elevated mitochondrial bioenergetics in mouse substantia nigra dopamine neurons as a vulnerability factor in Parkinson's disease

Authors: *C. PACELLI[1], N. GIGUERE[1], M.-J. BOURQUE[1], R. SLACK[2], L.-E. TRUDEAU[1]; [1]Departments of pharmacology and neurosciences, GRSNC, Fac. of Med., Univ. of Montreal, Montreal, QC, Canada; [2]Dept. of Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Parkinson's disease (PD) is accompanied by the loss of dopamine (DA) neurons within the mesencephalon. This loss is most extensive in the substantia nigra pars compacta (SNc), with considerably less cell loss in the closely located ventral tegmental area (VTA). Although converging evidence suggests that mitochondrial dysfunction plays a critical role in PD, it remains unclear why SNc DA neurons are so much more vulnerable to such dysfunction than other DA neurons. Enhanced expression of L-type calcium channels, involved in maintaining autonomous pacemaking in SNc neurons, has been proposed to play a key role in the vulnerability of these neurons due to its predicted large impact on cellular bioenergetics and associated oxidative stress. Another hypothesis is that SNc DA neurons have elevated energetic requirements because they possess an exceptionally large axonal arborization. Here we tested the hypothesis that SNc DA indeed have an elevated level of mitochondrial oxidative phosphorylation in comparison to VTA or olfactory bulb (OB) DA neurons. Using postnatal mouse DA neurons in primary culture, we quantified basal and maximal mitochondrial energetic metabolism using a Seahorse XF24 analyzer. We find that SNc cultures show considerably higher basal OCR (oxygen consumption rates) and higher maximal OCR compared to VTA or OB cultures. This was associated with a reduced respiratory reserve capacity (ratio between maximal and basal OCR), higher ATP content and higher reactive oxygen species production. Mitochondrial localization and density were also evaluated using viral-mediated overexpression of Mito-DsRed and confocal microscopy. SNc DA neurons were found to contain a higher density of mitochondria in their axonal compartment, but not in their somatodendritic compartment. Blocking L-type Ca(2+)channel with isradipine reduced basal OCR in SNc cultures. However, this did not abolish the difference between SNc and VTA cultures, arguing that other factors such as axonal arborization size are important determinants of basal bioenergetic expenditures in SNc DA neurons. Our results could contribute to a better understanding of the selective vulnerability of SNc DA neurons and other vulnerable neuronal populations in PD.

Disclosures: C. Pacelli: None. **N. Giguere:** None. **M. Bourque:** None. **L. Trudeau:** None. **R. Slack:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

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Program#/Poster#: 695.19/E15

Topic: C.03. Parkinson's Disease

Support: Natural Science Foundation of China 81102431

Natural Science Foundation of Jiangsu Province BK2011302

Title: Activated cathepsin L contributes to apoptotic death of SH-SY5Y cells exposed to 6-hydroxydopamine

Authors: *L. LI[1], L. GAO[1], L. XIAO[1], Z. LIANG[2];
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Abstract: Lysosome dysfunction may contribute to the etiology of neurodegenerative diseases, and autophagy is a common response to pathological damage in the process of neurodegeneration. In this study, we demonstrated that the neurotoxin 6-hydroxydopamine elicited increased autophagy in human neuroblastoma SH-SY5Y cells, as accessed by Lamp-1 and Lamp-2 immunostaining for Avs/autolysosomes, immunofluorescence and immunoblotting for the autophagy protein LC3 II /Atg 8. When treated with 6-OHDA, autophagy was associated with a transient increase in the intracellular expression and nuclear translocation of cathepsin L by a time-dependent manner in SH-SY5Y cells. Furthermore, we found that autophagy repression with 3-methyladenine accelerated the activation of caspase-3, therefore aggravated the cell apoptotic death induced by6-hydroxydopamine. Inhibition of cathepsin L with selective cathepsin L inhibitor Z-FF-FMK promoted autophagy by upregulation of LC3 II, and blocked the activation of caspase-3. Taking together, these results suggest that activation of autophagy may be primarily a protective process in SH-SY5Y cell death induced by 6-hydroxydopamine. Induction of cathepsin L could suppress autophagic cell-surviving pathway, contributing to cell apoptotic cascade in SH-SY5Y cells. These results highlight the potential role of lysosomal enzymes in the cross talk between autophagy and apoptosis, which should be considered in the therapeutic strategies for the treatment of pathology conditions associated with neurodegeneration.

Disclosures: L. Li: None. **L. Gao:** None. **L. Xiao:** None. **Z. Liang:** None.

Poster

695. Mechanisms of Cell Death and Dysfunction

Location: Halls A-C

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Program#/Poster#: 695.20/F1

Topic: C.03. Parkinson's Disease

Support: Neuro Canada

Krembil Foundation

Parkinson Society Canada

Title: Axonal arborization size as a key factor influencing the selective vulnerability of substantia nigra dopamine neurons in Parkinson's disease

Authors: ***N. GIGUERE**, C. PACELLI, C. SAUMURE, M.-J. BOURQUE, L.-E. TRUDEAU; Departments of pharmacology and neurosciences, GRSNC, Fac. of Med., Univ. De Montreal, Montreal, QC, Canada

Abstract: Parkinson's disease (PD) is accompanied by characteristic locomotor deficits that are thought to be due to the selective and progressive loss of dopamine (DA) neurons of the substantia nigra pars compacta (SNc) in the mesencephalon. Why SNc neurons are more vulnerable than DA neurons in other brain regions such as the closely located ventral tegmental area (VTA) is undetermined. We hypothesize that the main reason underlying this increased vulnerability is that SNc DA neurons have a much larger axonal arborization than VTA or other DA neurons, which would be associated with increased bioenergetic demands and increased oxidative stress. We tested this hypothesis by comparing the axonal and dendritic arborization of DA neurons cultured from the SNc, VTA or olfactory bulb (OB) of neonatal mice. Neurons were grown on an astrocytes monolayer for a period of 3 or 7 days, processed for TH immunocytochemistry and examined using confocal microscopy. Compatible with our hypothesis, we found that SNc DA neurons have a more elaborate axonal arborization than VTA DA neurons, with OB DA neurons having the least developed. The dendritic arborization of SNc DA neurons was also larger than that of OB neurons, but similar to that of VTA DA neurons. A close parallel was found between axonal arborization size and vulnerability to the DA neurons-selective neurotoxin MPP+: SNc DA neurons were much more vulnerable than VTA or OB DA neurons. A similar increased vulnerability was also observed for the mitochondrial complex I blocker rotenone, thus suggesting that the increased vulnerability to MPP+ was not due to differential expression of the DA transporter, through which MPP+ is uptaken. Our data show that SNc DA neurons have a much larger intrinsic axonal growth capacity than other DA neurons, which may place them at risk due to the increased bioenergetic demands that are predicted to be associated with this morphological phenotype. Our observation that SNc DA

neurons are also more sensitive to MPP+ and rotenone *in vitro* further argues that this increased vulnerability is cell-autonomous.

Disclosures: **N. Giguere:** None. **C. Pacelli:** None. **C. Saumure:** None. **M. Bourque:** None. **L. Trudeau:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.01/F2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R21 210210-0315

Title: A loss-of-function allelic series of the mouse C9ORF72 for studies of amyotrophic lateral sclerosis and frontotemporal dementia

Authors: ***L. P. BOGDANIK**, A. S. AUSTIN, C. R. LAMMERT, W. P. MARTIN, M. OSBORNE, C. LUTZ;
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Abstract: It was recently discovered that a GGGGCC hexanucleotide repeat expansion in a noncoding region of the C9ORF72 gene is the most frequent cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The mechanism by which this repeat expansion effects pathogenesis is as yet unclear; loss-of-function of the C9ORF72 gene, gain-of-function by toxic RNA aggregation and sequestration of RNA-binding proteins, and gain-of-function by aggregation of long dipeptide repeats are all modes that have been proposed and supported in cell and animal models. Newly-developed genome editing technologies, such as zinc-finger nucleases, TALENs, and CRISPR/Cas systems, have allowed us unprecedented control and efficiency in the induction of mutations in a variety of mouse strains. This, in contrast to previous techniques, confers the power to eliminate the confounding influence of different genetic backgrounds on phenotypes. Using sequence-specific zinc-finger nucleases, we have generated an allelic series of complete and transcript variant-specific knockouts and disruption events in the endogenous murine C9ORF72 ortholog in order to illuminate the effects of varying degrees of gene loss-of-function, and to create a mouse model of ALS/FTD suitable for downstream therapeutic research. We have characterized C57BL/6J mouse lines harboring deletions of three different lengths, in each of exons 1, 2, and 3. Here we show evidence of the

effects of these mutations on total expression, at both RNA and protein levels, as well as differential expression of two exclusive alternative transcript variants. More significantly, we have investigated the meaning of these genetic disruptions through histological analysis of central and peripheral nervous system tissues; motor unit number estimation by electromyography; and a battery of cognitive and behavioral phenotyping assays. The allelic series described here augment the resources maintained by the mutant mouse repository at the Jackson Laboratory, all available to the ALS community for further studies and preclinical testing.

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Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.02/F3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MESTD RS Project No. 41005

DAAD Project - Etablierung länderübergreifender akademischer Zusammenarbeit im Bereich Biologie

Title: Oxidative stress in the SOD1 G93A transgenic rat model of ALS: Subcellular details of glial activation and X-ray analysis of metal imbalance

Authors: *S. STAMENKOVIC[1], T. DUCIC[2], A. KRANZ[3], D. BATAVELJIC[4], V. SELAKOVIC[5], L. RADENOVIC[4], P. R. ANDJUS[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder characterized by death of neurons in cerebral cortex, brain stem and spinal cord. Most ALS cases are sporadic, while only 10% occurs in familial forms, among which 20% are caused

by mutations in the Cu, Zn-superoxide dismutase (SOD1). It is still unknown how these mutations lead to a toxic gain of function of SOD1 that causes motor neuron death, but it has been widely accepted that they increase susceptibility of SOD1 to form insoluble intracellular aggregations. It has been proposed that the mutant SOD1 is released to the extracellular space where it is then taken up by astrocytes and microglia leading to their activation and increased production of reactive oxygen species (ROS). Furthermore, loose binding of copper and zinc to the mutated SOD1 apoprotein may lead to disturbed tissue concentrations of these elements and contribute to increased oxidative stress. Having this in mind, we decided to check for markers of glial activation, oxidative stress and metal imbalance in the typical ALS target tissue - brain stem and in addition, based on our previous studies, in the hippocampus of the SOD1 G93A rat model of ALS. Immunohistochemistry for Iba1 and GFAP as markers of microglia and astrocytes, and SOD1, showed strong glial activation in these regions, and revealed pronounced intracellular aggregations of SOD1 in astrocytes. Analysis of 3D confocal images with IMARIS software revealed an activated morphology of both astrocytes and microglia in the brainstem of ALS rats, and confirmed the increased aggregation of SOD1 by astrocytes, while microglia showed signs of SOD1 excretion. In the hippocampus, astrocytes showed a similar activated profile, while microglia was unaffected. Examination of oxidative stress parameters by spectrophotometric biochemical assays detected increased presence of reactive oxygen and nitrogen species, increased index of lipid peroxidation, as well as decreased SOD1 and increased Mn SOD (SOD2) activity, in both investigated regions. Concurrently, investigations on the primary culture of cortical astrocytes with ROS production indicators, MitoSox red and H2DCDFA, showed increased ROS production after stimulation with hydrogen-peroxide in ALS compared to WT control. Finally, investigation of tissue elemental composition in the brainstem and hippocampus by X-ray fluorescence technique revealed increased copper accumulation in both regions of ALS animals, while presence of zinc was higher in the brainstem but lower in the hippocampus. All these results bring new incite to the oxidative stress mechanism and related glial and metal biomarkers in ALS.

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Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.03/F4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Georgetown University Funding

Title: Tyrosine kinase inhibitors modulate the immune profile in TDP- 43 overexpressing mice

Authors: *L. HEYBURN, M. HEBRON, S. SELBY, B. HARRIS, C. MOUSSA;
Georgetown Univ., Washington, DC

Abstract: Transactive response DNA-binding protein 43 kDa (TDP-43) is a widely expressed protein with DNA-, RNA-, and protein-binding properties. Mutations in the gene encoding TDP-43 are associated with neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Under some pathological conditions, TDP-43 undergoes several modifications including ubiquitination, cleavage into smaller fragments, insolubility, and accumulation in cytosolic and/or nuclear protein inclusions. TDP-43 has also been found in many other neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, and Parkinson's disease (PD). TDP-43 has been shown to interact with the PD-related protein Parkin. At the transcription level, TDP-43 binds to *Park2* mRNA, regulating its expression. However, Parkin post-translationally modifies TDP-43 by ubiquitinating it, perhaps mediating its cellular localization. The tyrosine kinase inhibitors (TKIs) Nilotinib and Bosutinib activate Parkin, leading to changes in TDP-43 levels and localization. In order to investigate how TDP-43 contributes to neuroinflammation, transgenic mice overexpressing human wild-type TDP-43 in neurons (TDP-43^{WT} mice) were used to measure markers of neuroinflammation compared to wild-type mice. Because TKIs activate Parkin and lead to alteration of TDP-43 levels and sub-cellular localization, where TDP-43^{WT} mice were given intraperitoneal injections of the TKIs Nilotinib, Bosutinib, or DMSO as a control every day for three weeks, and the level of neuroinflammatory markers was compared. Lysates were analyzed using a multiplex assay to measure 12 inflammatory markers: IL-1α, IL1β, IL-2, IL-4, IL-6, IL-10, IL-13, IFN-γ, TNF-α, MCP-1, RANTES, and VEGF. Bosutinib decreased IL-1α and increased IL-10, MCP-1 and VEGF in TDP-43^{WT} mice compared to mice injected with DMSO. Nilotinib increased IL-13 and decreased VEGF levels relative to mice injected with DMSO. Both drugs caused an increase in IFN-γ. These data indicate that the TKIs may modulate the immune response, leading to altered immune profiles that could either be beneficial or detrimental when TDP-43 is mislocalized.

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Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The Farber Family Foundation

Title: Fused in sarcoma (fus) regulates the expression and function of crucial presynaptic terminal proteins at mammalian neuromuscular junction- implications for fus-mediated als

Authors: *K. KRISHNAMURTHY, A. KIA, K. CHOMA, S. SHAMAMANDRI MARKANDAIAH, M. SHAHIDULLAH, I. LEVITAN, D. TROTTI, P. PASINELLI; NEUROSCIENCE, THOMAS JEFFERSON UNIVERSITY HOSPITAL FOR NEUROSCIENCE, PHILADELPHIA, PA

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that leads to fatal paralysis. About 10% of ALS cases are familial (fALS), and are associated with mutations in one or another of several genes, while the remainders are sporadic (sALS). FALS and sALS are clinically indistinguishable, suggesting that the different forms of the disease converge on common pathways. Destruction of the neuromuscular junction (NMJ) is a hallmark (and converging point) of all forms of ALS. One gene associated with fALS encodes the DNA/RNA binding protein Fused in Sarcoma (FUS), and FUS-related *Drosophila* models of ALS show early functional NMJ abnormalities as in human disease. In particular loss of the FUS homologue Cabeza disrupts the NMJ suggesting a role for FUS in formation of synapses at the NMJ. Previously we showed defects in synapse structure and function precede motor neuron degeneration in a *Drosophila* model of FUS ALS (Shahidullah et al., 2013). In this study, we investigated the molecular mechanism by which FUS regulates normal synaptic transmission at mammalian NMJ and how this function is compromised by ALS causative mutations in FUS. To understand the function of FUS at presynaptic terminals of NMJ we used siRNA mediated knock down and examined the expression of SV2, synaptotagmin and synaptophysin in cultured rat motor neurons. Single cell PCR analysis revealed a significant down regulation of SV2 and synaptotagmin genes and immunocytochemistry showed lower levels of these proteins. We are further examining the calcium influx through P/Q calcium channels in FUS knockdown neurons since synaptotagmin acts as a calcium sensor for synaptic vesicle release. Surprisingly, introduction of ALS causative mutant forms of FUS did not reduce the gene expression of SV2 but disrupted SV2 protein levels and synaptic vesicle release at mammalian NMJ. Based on the current findings, we hypothesize mutations in FUS result in novel gain of function that may impair either synaptic mRNA trafficking or protein synthesis at the motor neuron terminals. Experiments are underway to test this hypothesis.

Disclosures: **K. Krishnamurthy:** None. **A. Kia:** None. **K. Choma:** None. **S. Shamamandri Markandaiah:** None. **M. Shahidullah:** None. **I. Levitan:** None. **D. Trotti:** None. **P. Pasinelli:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.05/F6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The ALS gene FUS regulates macrophage activity in *Drosophila*

Authors: *J. MACHAMER, G. FULLER, T. LLOYD;
Johns Hopkins, Baltimore, MD

Abstract: The progression of Amyotrophic Lateral Sclerosis (ALS) has been shown to depend upon nonneuronal cells including microglia, astrocytes, and oligodendrocytes; however, the molecules that mediate these effects are unknown. Mutations in the gene FUS cause ~5-10% of inherited ALS and cause aggregation and mislocalization of FUS protein from the nucleus to the cytoplasm. However, mutant FUS does not aggregate in motor neurons of *Drosophila*, but we find that expressing mutant forms of FUS in one specific neuronal subtype, dendritic arborization (da) neurons, results in the cytosolic mislocalization and aggregation of FUS, as seen in patients and mouse models of FUS-mediated ALS. Intriguingly, loss of FUS function in these da neurons causes macrophages to adhere to and cluster around larval segmental nerves. This abnormal association of macrophages with glial cells leads to swelling of the blood-nerve barrier and disruption of motor axon integrity. These data demonstrate that ALS-causing mutations result in mislocalization and aggregation of FUS within da neurons, and that loss of FUS function within these neurons results in the activation of macrophages that adhere to glia and disrupt nerve morphology. These findings indicate a novel cell nonautonomous function of FUS in neuronal regulation of macrophage activity, which when disrupted could contribute to the pathogenesis of ALS.

Disclosures: **J. Machamer:** None. **G. Fuller:** None. **T. Lloyd:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: the Korea Health technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A120443).

Title: ALS-linked FUS-positive stress granules are regulated by autophagy

Authors: M.-H. JUN, H.-H. RYU, K.-J. MIN, *J.-A. LEE;
Biotech., Hannam Univ., Dajeon, Korea, Republic of

Abstract: Mutations in Fused-in-Sarcoma (FUS), a DNA/RNA binding protein, have been associated with familial amyotrophic lateral sclerosis (fALS), which is a subset of frontotemporal lobar dementia (FTLD). However, the role of autophagy in regulation of FUS-positive stress granules (SGs) and aggregates remains unclear. We found that the ALS-linked FUS(R521C) mutation causes accumulation of FUS-positive SGs under oxidative stress, leading to disruption of release of FUS from SGs in cultured neurons. Autophagy controls the quality of proteins or organelles; therefore, we checked whether it regulates R521C-positive SGs. Interestingly, R521C-positive SGs were colocalized to RFP-LC3-positive autophagosomes. Furthermore, FUS-positive SGs accumulated in atg5-/- MEFs (mouse embryonic fibroblasts) and in autophagy-deficient neurons. However, R521C expression did not significantly impair autophagic flux. Moreover, autophagy activation with rapamycin reduced the accumulation of FUS-positive SGs in an autophagy-dependent manner. Rapamycin further reduced neurite fragmentation in neurons expressing mutant FUS under oxidative stress. Overall, we provide a novel pathogenic mechanism of ALS associated with a FUS mutation under oxidative stress, as well as therapeutic insight regarding FUS pathology associated with excessive SGs.

Disclosures: **M. Jun:** None. **H. Ryu:** None. **J. Lee:** None. **K. Min:** None.

Poster

696. Motor Neuron Disease and Animal Models

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Department of Veteran Affairs

Title: A directed genetic screen reveals loss of rad23 as a suppressor of neurodegeneration

Authors: *A. JABLONSKI[1], T. LAMITINA[1], N. F. LIACHKO[2], J. LIU[3], J. MOJSILOVIC-PETROVIC[4], B. KRAEMER[2], J. WANG[3], R. G. KALB[4,1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Geriatric Res. Educ. and Clin. Ctr., Univ. of Washington, Seattle, WA; [3]Biochem. and Mol. Biol., Johns Hopkins Univ., Baltimore, MD; [4]Res. Inst., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Accumulation of misfolded proteins is seen in neurodegenerative diseases and cellular processes that ensure correct protein folding may modify proteotoxicity and other age-related defects in neurodegeneration. One protein quality control mechanism is ER- associated degradation (ERAD). Improperly folded cell-surface or secreted proteins are retrotranslocated from the ER for ubiquitin-dependent degradation. Several studies have linked the dysfunction of ERAD and the ubiquitin-proteasome system (UPS) to neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). Our goal was to identify modifiers of proteotoxicity by using the genetic tool, *Caenorhabditis elegans*. *C. elegans* engineered to express a mutant version of TDP-43 implicated in familial ALS (M337V) (mutTDP-43) in neurons display locomotor deficits and motor neuron death. Using a directed genetic screen approach focusing on genes in the ERAD and UPS pathways, we identified loss of *rad23* as a suppressor of the mutTDP-43 locomotor deficit. We found that this effect could be rescued by overexpression of RAD23 in the nervous system alone. RAD23 functions in nuclear excision repair and in delivering ubiquitinylated substrates to the proteasome. Interestingly, loss of *rad23* conferred a resistance to proteotoxic stressors, but a specific deficit in UV sensitivity. Knockdown of the 2 orthologs of *rad23* in mammalian motor neurons also suppressed TDP-43 toxicity. We found that loss of no other gene in RAD23's known ERAD and NER pathways suppressed the mutTDP-43 locomotor deficit. We therefore investigated whether or not loss of *rad23* improved protein homeostasis. We found that loss of *rad23 in vivo* or *in vitro* decreased mutTDP-43 steady state abundance and insolubility, but did not decrease *rad23* mRNA abundance. This effect on protein abundance could be blocked *in vitro* by inhibition of the proteasome. In worms with mutSOD-YFP (mutant (G85R) SOD-1) expressed in the nervous system, we were able to show loss of *rad23* increased the recovery of mutSOD measured by fluorescence recovery after

photobleaching, suggesting loss of *rad23* increases mutSOD solubility as well. We also found that knockdown of RAD23 accelerated mutTDP-43 turnover when protein synthesis was inhibited. In sum, this work suggests that RAD23 could be a new therapeutic target in ALS. In a human ALS case, we were able to find a strong abnormal accumulation of cytoplasmic RAD23. It also suggests that stabilizing factors of protein aggregates could be a new target in the treatment of neurodegeneration. Future work will focus on if loss of *rad23* could be protective in other forms of neurodegeneration.

Disclosures: A. Jablonski: None. **T. Lamitina:** None. **N.F. Liachko:** None. **J. Liu:** None. **J. Mojsilovic-Petrovic:** None. **B. Kraemer:** None. **J. Wang:** None. **R.G. Kalb:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.08/F9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Christa Lorenz Foundation

Title: Expression of iron-related proteins in the g93a-sod1 model of amyotrophic lateral sclerosis

Authors: *C. MAWRIN, M. HADZHIEVA, E. KIRCHES;
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Abstract: *Objective:* To elucidate a potential increase of cellular iron and a dysregulation of genes related to iron import, storage and mitochondrial iron metabolism in cells harbouring the G93A mutation of SOD1, associated with familial ALS. *Methods:* Determination of total cellular and mitochondrial iron, qPCR-quantification of mRNAs of transferrin receptor 1 (TfR1), ferritin, divalent metal transporter 1 (DMT1), mitoferins (Mfrn 1 and 2), frataxin (FXN) and iron sulfur cluster scaffold protein (IscU) in neuroblastoma cells stably transfected with SOD1-A93G or SOD1-wt, under various concentrations of iron in the culture medium and in presence or absence of external oxidative stress. *Results:* An increase of total iron content was observed in the G93A cells as compared to controls, which would be in accordance with a simultaneous increase of TfR1 and DMT1. Experiments with the iron chelator deferoxamine revealed a normal reaction of both cell lines to iron-depletion, i.e. TfR1 upregulation, suggesting an intact function of the IRE/IRP regulatory machinery. The expression levels of Mfrn 1 and 2, FXN and IscU were also increased significantly in the G93A cells. This suggested a higher mitochondrial import and

utilization of iron for the two main biosynthetic pathways, i.e. heme and ISC biogenesis. Because the expression of these transcripts was further enhanced by a ROS-inducing retinoic acid treatment (increased DCF fluorescence), ROS may participate in the dysregulation of these genes, supporting a feedback between enhanced iron levels and ROS. Conclusions: The model feeds the hypothesis that iron and altered iron-metabolism participate in the disease process of ALS. It further suggests that oxidative stress may not solely be a result of increased iron (Fenton reaction), but may vice versa help to establish iron overload and accelerated mitochondrial iron metabolism.

Disclosures: C. Mawrin: None. **M. Hadzhieva:** None. **E. Kirches:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS034100

NIH Grant NS065895

Title: Voltage-gated calcium channels are abnormal in cultured spinal motoneurons in the G93A-SOD1 transgenic mouse model of ALS

Authors: *Q. CHANG[1], L. J. MARTIN[2];
[1]Pathol, Johns Hopkins Univ., Baltimore, MD; [2]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of motoneurons. Hyperexcitability and excitotoxicity have been implicated in the early pathogenesis of ALS. Studies addressing excitotoxic motoneuron death and intracellular Ca^{2+} overload have mostly focused on Ca^{2+} influx through AMPA glutamate receptors. However, intrinsic excitability of motoneurons through voltage-gated ion channels may also have a role in the neurodegeneration. In this study we examined the function and localization of voltage-gated Ca^{2+} channels in cultured spinal cord motoneurons from mice expressing a mutant form of human *superoxide dismutase-1* with a Gly93→Ala substitution (G93A-SOD1). Using whole-cell patch-clamp recordings, we showed that high voltage activated

Ca^{2+} currents are increased in G93A-SOD1 motoneurons, but low voltage activated Ca^{2+} currents are not affected. G93A-SOD1 motoneurons also have altered persistent Ca^{2+} current mediated by L-type Ca^{2+} channels. Quantitative single-cell RT-PCR revealed higher levels of Ca1a, Ca1b, Ca1c, and Ca1e subunit mRNA expression in G93A-SOD1 motoneurons, indicating that the increase of high voltage activated Ca^{2+} currents may result from upregulation of Ca^{2+} channel mRNA expression in motoneurons. The localization of the Ca1B N-type and Ca1D L-type Ca^{2+} channels in motoneurons was examined by immunocytochemistry and quantitative confocal microscopy. An increase of Ca1B channels was observed on the plasma membrane of soma and dendrites of G93A-SOD1 motoneurons. In contrast, Ca1D channels on the plasma membrane of dendrites of G93A-SOD1 motoneurons were lower than that of control motoneurons. Our study suggests that alterations in voltage-gated Ca^{2+} channels contribute to Ca^{2+} overload in motoneurons in the disease process of ALS.

Disclosures: Q. Chang: None. **L.J. Martin:** None.

Poster

696. Motor Neuron Disease and Animal Models

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NS060926

NS077863

Title: Electrophysiological properties of medial motoneurons in a mouse model of mild SMA

Authors: *K. A. QUINLAN[1], C. CHRZASTOWSKI[2], C. J. HECKMAN[1], C. J. DIDONATO[2]; [1]Physiol., Northwestern Univ. Feinberg Sch. of Med., CHICAGO, IL; [2]Pediatrics and Human Mol. Genet., Lurie Children's Hosp. Northwestern Univ., Chicago, IL

Abstract: Spinal muscular atrophy (SMA) is a pediatric autosomal recessive neuromuscular condition caused by low levels of the survival motor neuron (SMN) protein. Patients range in disease severity from type I (most severe, neonatal mortality) to type III (mild, affects motor control in older children and adults). Newly created mouse models for mild SMA recapitulate symptomology of human patients, including motor axon loss and motor control deficits. The disease arises from deficient levels of the ubiquitously-expressed SMN protein, yet it results in

the specific loss of motoneurons. This loss is preceded by dramatic changes in motoneuron excitability and loss of afferent inputs. In our current work, we compare the electrophysiological properties of mild SMA mice at just over one week of age (P8-9), long before any overt symptoms appear (lifespan ~ 2 years). Whole cell patch clamp was performed on large neurons in the medial motoneuron pools in 350 um thick transverse spinal cord slices from lower thoracic to upper lumbar regions (T10 - L2). Properties were analyzed in voltage clamp (for persistent inward currents) and in current clamp (for action potential / after hyperpolarization characteristics, frequency-current relationships, etc). In the mild SMA mouse, the etiology of motoneuron loss and time course of electrophysiological changes vs. functional losses can be much more clearly delineated, revealing new insights into disease progression and potential windows for treatment before motoneurons and /or motor axons are lost.

Disclosures: K.A. Quinlan: None. **C.J. Heckman:** None. **C.J. DiDonato:** None. **C. Chrzastowski:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.11/F12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Targeted ablation of myelinating Schwann cells enhances disease severity in SOD1G93A mice

Authors: *R. K. SHEEAN, J. A. STRATTON, T. D. MERSON, B. J. TURNER;
Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia

Abstract: Background Mutant SOD1 action within non-neuronal cells is implicated in damage to motor neurons in genetic forms of ALS. Astrocytes, microglia and oligodendrocytes drive progression in mutant SOD1 mice, while the role of Schwann cells is less clear. Schwann cells, the myelinating glial cells of the peripheral nervous system, are intimately associated with motor neurons and are vital for nerve conduction, axonal development, transport and support. The role of Schwann cells in ALS pathogenesis is unequivocal. Selective removal of mutant SOD1 from Schwann cells accelerates disease progression, while restricted expression of mutant SOD1 in Schwann cells is not harmful to mice. To resolve the contribution of Schwann cells to ALS, we generated double transgenic mutant SOD1;MBP-DTR mice that allows selective elimination of myelinating Schwann cells. Objectives To investigate the effect of myelinating Schwann cell

depletion on disease onset, progression and spinal cord and peripheral nerve pathology in SOD1G93A mice. Methods SOD1G93A mice were crossed with novel transgenic MBP-DTR mice which express diphtheria toxin receptor (DTR) driven by the myelin basic protein (MBP) promoter. Exogenous administration of diphtheria toxin (DT) to MBP-DTR mice results in selective ablation of 25% of myelinating Schwann cells that is sub lethal. Double transgenic SOD1G93A;MBP-DTR mice and control genotypes SOD1G93A, MBP-DTR and wild-type (WT) were injected with DT (10μg/kg, ip) at presymptomatic age (P60) and disease onset and progression was determined using rotarod and grid test performance and survival was assessed. Spinal cords and sciatic nerves were analysed by immunohistochemistry and electron microscopy for motor neuron and axonal counts, Schwann cell apoptosis and myelination. Results Administration of DT provoked hindlimb weakness and wasting in MBP-DTR mice which peaked at 22 days post injection (P82) followed by rapid recovery by 28 days post injection (P88). This resulted from 25% depletion of Schwann cells and demyelination in sciatic nerves. Double transgenic SOD1G93A;MBP-DTR mice showed increased severity of muscle weakness and wasting at peak symptoms and Schwann cell loss and demyelination. Administration of DT to SOD1G93A or WT mice did not elicit symptoms or Schwann cell death as mice are naturally resistant to diphtheria. Discussion and conclusions Our data demonstrate that DT-induced myelinating Schwann cell ablation and resulting motor dysfunction is enhanced by mutant SOD1 expression in Schwann cells. This suggests that mutant SOD1 damage to Schwann cells sensitises them to death in this toxin-induced model.

Disclosures: R.K. Sheean: None. **J.A. Stratton:** None. **T.D. Merson:** None. **B.J. Turner:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NEI K08-EY021520

NEI core grant P30EY005722

Butler Pioneer Award

Title: Motor deficits in a novel optineurin mouse for amyotrophic lateral sclerosis

Authors: *C. MCKEE[1], H. BOMZE[1], T. RHODES[2,3], C. MEANS[2,3], R. RODRIGUIZ[2,3], W. WETSEL[2,3,4], H. TSENG[1];
[1]Ophthalmology, [2]Psychiatry and Behavioral Sci., [3]Mouse Behavioral and Neuroendocrine Analysis Core Facility, [4]Cell Biol. and Neurobio., Duke Univ., Durham, NC

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease in which progressive loss of motor neurons leads to declining motor function and eventually death. Many genetic mutations have been identified in familial ALS (e.g., SOD1, ANG, TARDP, TDP-43, FUS), but they are associated with only a small number of ALS patients. The optineurin gene (OPTN), responsible for primary open angle glaucoma, has been associated recently also with familial ALS. Many ALS-associated mutations in OPTN are located in key regions of the gene that would disrupt this gene's interactions with other proteins. Thus, we hypothesize that loss of normal optineurin function may lead to motor behavioral deficits, and we tested this hypothesis by generating a novel conditional optineurin knockout mouse using Cre-Lox recombination technology. Homozygous mice with genetic deletion of optineurin were tested for motor strength, coordination, learning, and general motor activity in behavior assays, and their responses were compared to nontransgenic C57BL/6 controls and CMV-Cre controls (n = 12 animals for each genotype). Statistically-significant deficits were found in the rotorod and foot-fault tests, but not in other behavioral assays. Optineurin knockout mice were deficient on the steady-speed rotorod test relative to the control groups, but showed intact motor learning on the accelerating rotorod and foot-fault tests. Taken together, our data indicate that genetic deletion of optineurin in mice results in specific motor deficits and suggest that these mice will be useful as a model for studying ALS.

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Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.13/G2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Novel sensitive kinematic gait analysis reveals early motor impairment in G93A SOD1 mice of amyotrophic lateral sclerosis

Authors: *J. OKSMAN[1], T. HEIKKINEN[1], T. BRAGGE[1], T. AHTONIEMI[1], O. KONTKANEN[1], A. STEPHAN[2], R. RUTTER[2], A. NURMI[1];
[1]Charles River Discovery Res. Services Ltd, Kuopio, Finland; [2]Neural Pathways DPU, Neurosciences TAU, GSK, Singapore, Singapore

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that progressively leads to paralysis and death. It is caused by the loss of motoneurons especially in the spinal cord, and 15-20% of familial ALS cases are linked to a mutation of the copper/zinc superoxide dismutase (Cu/Zn SOD, SOD-1) gene. The aim of this study was to characterize phenotypical deficits in a transgenic mouse model G93A-SOD1 mice by using kinematic gait analysis, with a novel automated high precision movement analysis system. Gait properties were analyzed in freely moving G93A transgenic (n=15) male mice and their wild-type (n=15) littermates. The gait was recorded with a high speed camera, imaged and analyzed simultaneously from three spatial dimensions, via comprehensive kinematic algorithms. Each point (paws) of movement trajectory was calculated as a change in coordinates and used for data analysis. Parameters that were analyzed included kinematic gait properties at the age of 8, 11, 14 and 16 weeks respectively. Gait in G93A mice deteriorates progressively, with the most pronounced changes occurring in the coupling (parameters) from 80 days of age. Homolateral coupling increases while diagonal coupling, which is the preferred trot-like gait in WT healthy mouse, respectively decreases. This change was evident from 11 weeks of age (~80 days) onwards until 16 weeks. In addition, gait deficits appear primarily in hind limbs, with defects becoming readily apparent at 80 days of age. This is in accordance with the progression of paralysis, which starts typically from the hind limbs. For comparison, classical behavioral tests such as rotarod and grip strength reveal typical phenotypic differences in motor performance somewhat later at the age of 13 weeks (~90 days). Additionally, hind limb position in gait cycle changes towards the medial axis, possibly reflecting on the fact that an increased rigidity in these mice exists while normal mice have comparatively greater flexibility in the lateral dimension. In accordance with changes in step width in hind limbs, the swing time in hind limbs increases progressively from 14 weeks of age (~100 days). This study shows that kinematic gait analysis may provide a more sensitive tool to study progressive phenotypes associated with certain CNS diseases, and offers an efficient tool to study treatment effects on fine motor symptoms, with earlier detection and enhanced sensitivity compared to classical motor behavior tests.

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Poster

696. Motor Neuron Disease and Animal Models

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NINDS NS066888

Title: Generation and characterization of models for Amyotrophic Lateral Sclerosis in *C. elegans*

Authors: ***S. N. BASKOYLU**, A. C. HART;
Neurosci., Brown Univ., Providence, RI

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive disease of the nervous system that causes motor neuron degeneration, paralysis and death. Multiple genes involved in RNA homeostasis have been linked to disease, including transactive response DNA binding protein (TDP-43) and fused in sarcoma (FUS). Both TDP-43 and FUS pathology is marked by aggregation of the affected protein in the cytoplasm with its concurrent depletion from the nucleus, suggesting a shared disease mechanism. To investigate the role of TDP-43 and FUS dysfunction in disease, we are constructing precise ALS models in *C. elegans* that express conserved patient alleles in *C. elegans* TDP-43 and FUS orthologous proteins TDP-1 and FUST-1, respectively. We plan to characterize the animal models in well-described behavioral assays. Our goal is to identify ALS genetic modifiers in our precise *C. elegans* models and to use this information to organize ALS linked genes in functional pathways pertinent to disease.

Disclosures: S.N. Baskoylu: None. **A.C. Hart:** None.

Poster

696. Motor Neuron Disease and Animal Models

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: GM103418

HD57850

HD02528

Title: Effects of tongue force training on bulbar motor function in SOD1-G93A rats

Authors: *J. A. STANFORD[1], D. MA[2], J. M. SHULER[2], T. TOMOHIRO[2], T. SUDHEER[2], H. NISHIMUNE[2];
[1]Dept Molec & Integrat Physiol, Univ. Kansas Med. Ctr., KANSAS CITY, KS; [2]Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Protecting neuromuscular junction (NMJ) innervation and maintaining muscle strength are critical therapeutic goals in Amyotrophic Lateral Sclerosis (ALS). SOD1-G93A rats model ALS in that they exhibit a presymptomatic stage followed by neuromuscular denervation, progressive muscle weakness and atrophy, and finally paralysis. Studies examining NMJ protection in preclinical models of ALS have typically focused on spinal deficits. Accordingly, we have found that isometric forelimb strength training protects against NMJ denervation in forelimb muscles of SOD1-G93A rats. In order to extend preclinical testing to bulbar deficits, we previously reported orolingual motor deficits in SOD1-G93A mice and rats using a task that measures tongue force and tongue motility (licking speed) as animals lick water from a force-sensing disc. In SOD1-G93A rats, orolingual motor deficits were especially prevalent in females, which is consistent with clinical findings in human ALS. The goal of the current study is to determine whether tongue strength training protects against these deficits in female SOD1-G93A rats. We are testing SOD1-G93A and wildtype rats in the licking task during 2-min morning sessions under minimal (1-g) force requirements (5 days/week). Half of the rats in both groups are being placed in the testing chambers again in the afternoon, but are required to produce greater tongue forces (10-g) during 6-min sessions (i.e., the strength training group). Body weight is being recorded 5 days/week and forelimb grip force is being measured 2 days/week in all rats. Testing will continue until end stage, which will be defined for each rat as the onset of limb paralysis. Orolingual motor measures during the testing sessions will be analyzed as a function of genotype (SOD1-G93A vs wildtype) and training group. We will also analyze these measures as a function of genotype during training sessions. We will then collect genioglossus (tongue protruder) muscles and analyze NMJs for denervation. Findings will be discussed in the context of tongue strength training as an intervention for bulbar motor deficits and NMJ protection in ALS.

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Poster

696. Motor Neuron Disease and Animal Models

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Title: Interaction of PCBP1 with mutant SOD1 in transgenic ALS pig brains

Authors: *G. WANG;
Human Genet., Emory Univ., Atlanta, GA

Abstract: Interaction of PCBP1 with mutant SOD1 in transgenic ALS pig brains Guohao Wang[1,2,] Huaqiang Yang[3] Shihua Li[2] Liangxue Lai[3] Xiao-Jiang Li[1,2] [1]Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia, USA; [2]State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; [3]Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China Mutations of the SOD1 gene cause familial amyotrophic lateral sclerosis (ALS) in human. It remains unknown whether large animal models of ALS mimic more pathological events seen in ALS patients via novel mechanisms. We have generated transgenic pigs expressing mutant G93A hSOD1, which show hind limb motor defects and nuclear accumulation as well as ubiquitinated nuclear aggregates. These nuclear aggregates were not reported in transgenic SOD1 mouse models but were seen in some ALS patient brains, suggesting that species differences contribute to differential pathology in small and large animal models of ALS. To investigate the mechanism underlying this species-dependent pathology, we used GST-pulldown to identify the pig brain proteins that can bind mutant SOD1. Our results revealed that SOD1 binds PCBP1, a nuclear poly(rC) binding protein, in pig brain, but not in mouse brain. PCBP1 is also colocalized with nuclear SOD1 aggregates in transgenic ALS pig brains. Our findings suggest that the SOD1-PCBP1 interaction accounts for nuclear SOD1 accumulation and that species-specific targets are key to ALS pathology in large mammals and in humans. Supported by *the National Institutes of Health (NS036232, NS041669 and NS045016).*

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Poster

696. Motor Neuron Disease and Animal Models

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ALS Association Grant 14DUYT

ALS Therapy Alliance Grant 2013-F-052

NSF GRFP

Title: Characterization of bacTRAP transgenic mice that label cortical projection neuron populations to study ALS degeneration

Authors: *E. F. SCHMIDT[1], M. V. MOYA[1], C. E. SFERRAZZA[1], K. L. MCGUIRE[1], S. B. PICKETT[1], N. HEINTZ[1,2];
[1]Lab. Mol Biol, Rockefeller Univ., NEW YORK, NY; [2]Howard Hughes Med. Inst., The Rockefeller Univ., New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating and fatal disease targeting the motor pathways of the central nervous system. It is characterized by the selective degeneration of the “lower” motor neurons in the brainstem and spinal cord and “upper” motor neurons in the cerebral cortex. An upper motor neuron component is generally required to distinguish clinical cases of ALS from other motor neuron diseases. ALS afflicts these discrete cell populations despite the fact that the majority of genes linked to ALS (including SOD1 and TDP-43) are ubiquitously expressed. To fully understand the causes of ALS and to develop better therapies, it is imperative to investigate the molecular pathologies occurring specifically in vulnerable cells. The majority of preclinical research using rodent models has focused on the pathology of spinal cord motor neurons with only few reports documenting cortical deficits. This likely reflects the difficulty in distinguishing upper motor neurons from other pyramidal cell types due to the heterogeneous composition of the cortex. Therefore a large gap exists in our understanding of the molecular mechanisms and pathways underlying upper motor neuron degeneration. Here, we describe transgenic tools that label distinct genetically-defined cortical projection neuron populations. We used bacterial artificial chromosome (BAC) promoters to drive the expression of an EGFP-tagged ribosomal protein (EGFP-Rpl10a) in corticospinal projecting "upper" motor neurons, which are vulnerable to degeneration in ALS, as well as non-vulnerable corticothalamic cells. The EGFP-Rpl10a transgene allows for Translating Ribosome Affinity Purification (TRAP) molecular phenotyping of these cells types during disease progression. Retrograde labeling with cholera toxin beta subunit to identify axonal targets, assessing the distribution of

cell bodies in the cortical plate, and immunolabeling with known markers revealed that each of these transgenic lines labeled anatomically distinct cell populations. Further, analysis of TRAP mRNA from each cell type by RNA-seq demonstrated that these cells are molecularly discrete from one another as well. Therefore, these novel bacTRAP transgenic mice will be valuable tools for studying cell-type specific degeneration in ALS disease models.

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Poster

696. Motor Neuron Disease and Animal Models

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Medical Research Council Core Support

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Title: Automated home-cage assessment in SOD1 mice uncovers early sex-dependent deficits in voluntary wheel running and cognitive timing ability

Authors: *S. MANDILLO[1], L. GARBUGINO[1], S. MAGGI[2], I. HEISE[3], E. GOLINI[1], G. P. TOCCHINI-VALENTINI[1], P. M. NOLAN[3], V. TUCCI[2];
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Abstract: Motor and cognitive ability evaluations are crucial in assessing mouse models of neurodegenerative disease. We recently validated automated home-cage based running-wheel and interval timing tasks (TSE Systems) that enable continuous monitoring of motor and cognitive performance without handler interference, features that are desirable in longitudinal studies. To study how deficits vary with respect to sex, age of onset, rate of progression and severity of symptoms, we investigated these parameters in a genetic model of Amyotrophic Lateral Sclerosis (ALS), Tg(SOD1G93A)dl1/GurJ (SOD1). We challenged male and female

mice of different ages on one or multiple 3-weeks sessions to verify the effect of voluntary wheel running on the disease progression, motor function and survival. Male and female SOD1 mice differed in survival and disease progression depending on wheel running experience. Repeated 3-weeks wheel running sessions improved motor function but shortened survival in male SOD1 mutant mice. SOD1 mutants were also tested in an automated home-cage apparatus to evaluate cognitive timing ability and its circadian regulation. Error rate at discrete time points across the light-dark cycle was altered in mutants as was their ability to discriminate between short and long intervals. Females showed greater impairments than males. Wheel-running and cognitive difficulties were observed in SOD1 mutants earlier than those detected using conventional tasks, such as the Rotarod, making these home-cage automated systems reliable tools to uncover deficits at pre-symptomatic stages in models of neurodegenerative disease.

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Poster

696. Motor Neuron Disease and Animal Models

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ALS Therapy Alliance Grant 2013-F-052

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Title: Molecular phenotyping of "upper" motor neuron degeneration in the SOD1-G93A mouse model

Authors: *M. V. MOYA[1], C. E. SFERRAZZA[1], K. L. MCGUIRE[1], S. B. PICKETT[1], N. HEINTZ[1,2], E. F. SCHMIDT[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease characterized by specific death of “lower” motor neurons in the spinal cord and “upper” motor neurons in the cortex. Because the mutations that have been linked to ALS tend to be ubiquitously expressed, it is imperative to study the distinguishing molecular characteristics that

make motor neurons selectively vulnerable to degeneration. The focus of many studies of ALS mouse models has been on the degeneration of the spinal motor neurons, perhaps because selective targeting of upper motor neurons over non-vulnerable pyramidal neurons in the cortex has proven difficult. But in order to fully elucidate the causes of ALS and develop targeted therapies, it is necessary to investigate the mechanisms of degeneration in "upper" motor neurons as well. For this reason, we have set out to genetically and molecularly characterize the progression of "upper" motor neuron degeneration in the SOD1-G93A mouse model. In addition to staining for known markers of degeneration, we employed the Translating Ribosome Affinity Purification (TRAP) technique to quantitatively determine gene expression profiles of upper motor neurons in the SOD1-G93A mouse model before and after onset of ALS symptoms. We took advantage of the Gprin3 BAC promoter to selectively express the EGFP-tagged ribosomal subunit L10a (EGFP-Rpl10a) TRAP transgene in a subpopulation of "upper" motor neurons. We performed TRAP on cortex from Gprin3-bacTRAP mice crossed to SOD1-G93A mice and compared these results to the non-vulnerable layer 6 corticothalamic Ntsr1-bacTRAP cells. RNA-seq analysis of TRAP mRNA identified genes whose expression levels were changed in the "upper" motor neurons of the ALS mice. We also compared the results of these groups to results from the same cells at pre-symptomatic stages of the disease, which allowed us to identify early markers of degeneration. In order to visualize some of these genes associated with degeneration, we immunohistochemically stained for the protein products in Gprin3-bacTRAP SOD1-G93A brains. By staining for the same proteins in pre-symptomatic and post-symptomatic tissue, we have begun to establish mechanisms of degeneration in the "upper" motor neurons that begin before symptom onset. These motor neuron-specific degenerative markers will allow us to further dissect mechanisms of selective cell death in ALS and may yield future targets for therapies.

Disclosures: **M.V. Moya:** None. **C.E. Sferrazza:** None. **K.L. McGuire:** None. **S.B. Pickett:** None. **N. Heintz:** None. **E.F. Schmidt:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 696.20/G9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: AG000944 to H. CAI

AG000928 to H. CAI

Title: Loss-of-function of the microtubule binding domain of p150glued is sufficient to trigger spinal motor neuron degeneration

Authors: *J. YU, C. LAI, L. SUN, C. XIE, H. SHIM, H. CAI;
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Abstract: Dynein/dynactin motor protein complex mediates the retrograde transport of organelles, vesicles and molecules in neurons. P150glued, encoded by Dctn1 gene, is the largest component of dynactin complex. Neurons contain p150glued, the full-length protein with the microtubule binding domain (MTBD), and p135, the short form that lacks the MTBD due to alternative splicing. The G59S missense mutation at MTBD of p150glued has been associated with a slowly progressive, autosomal dominant form of lower motor neuron disease (MND) without sensory symptoms in a North American family. Our previous work showed that P56S knock-in mouse displayed spinal motor neuron loss and gait abnormality after 10 months of age. However, whether P56S causes neurodegeneration by a loss-of-function or gain-of-toxicity mechanism is not yet established. In the present study, by utilizing Cre-loxP recombination technology, we generated Dctn1flox/flox/Thy1-Cre mice (conditional knock-out, cKO), in which the expression of p150glued is specifically knocked down in post-mitotic neurons at cerebral cortex, hippocampus, striatum, midbrain, cerebellum, brain stem and spinal cord, as compared with Dctn1flox/flox mice (littermate control, Ctrl). Interestingly, the expression level of p135 was increased in cKO mice, while the expression of other dynactin subunits, such as p62, dynamitin, Arp1 and p25 did not change. Additionally, co-immunoprecipitation and sucrose density gradient centrifugation experiments demonstrated that p135 and other dynactin subunits remained to form intact protein complex with dynein family proteins, indicating that the MTBD of p150guled was not required for the assembly of dynein/dynactin complex. Next, we examined motor behavior of mice by open-field and rotarod tests, and found that cKO mice developed obvert defects in motor coordination and rearing movement after 12 and 18 months of age, respectively. Moreover, unbiased stereological analysis showed 22% loss of alpha motor neurons in the lumbar spinal cord of 18 months old cKO mice, whereas no obvious neurodegeneration was found in cerebral cortex, hippocampus and cerebellum. Notably, although no spinal motor neuron loss was observed in 9-month-old cKO mice, significantly increased RAB24 (autophagosome marker) and LAMP2 (lysosome marker) expression was detected in the spinal cord, implying an alteration of authophagy/lysosome pathway may occur prior to the neurodegeneration in these mice. Taken together, our findings reveal an important role of p150glued MTBD in the survival of spinal motor neurons during aging, and suggest a loss-of-function mechanism of p150glued in the pathogenesis of MND.

Disclosures: J. Yu: None. **C. Lai:** None. **L. Sun:** None. **C. Xie:** None. **H. Shim:** None. **H. Cai:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: UAMS Startup Funds

NIGMS IDeA P20 GM103425-10

Title: Development and characterization of mutant human profilin1 transgenic mice as new model for als

Authors: *S. YADAV, M. KIAEI;
Neurobio. & Dev. Sci., Univ. of Arkansas For Med. Sci., Little Rock, AR

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that mainly affects motor neurons in cortex, brainstem and spinal cord. Recently, five mutations in profilin1 (*PFN1)* gene (ALS18) were linked to a subpopulation of fALS patients which had none of the previously known mutated genes in fALS (Wu et al., 2012). Whether PFN1 mutations in ALS patients is a cause of ALS, remain unknown. Human *PFN1* mapped in chromosome 17p13.3 and deletion in this locus is associated with Miller-Dieker syndrome, a cause of type I Lissencephaly (Miller 1963). Identification of *PFN1* mutation in ALS patients with 10 years earlier average age of onset than other ALS patients, and common clinical limb onset suggest its involvement but doesn't confer that it is the cause. We developed a transgenic mouse model to overexpress human PFN1 with a mutation found in human ALS patients and analyzed the animals for ALS-like phenotypes and study the mechanism(s) for PFN1 neurotoxicity. We have successfully created three lines of transgenic mice overexpressing mutant human PFN1, High (H), Medium (M) and low (L) expressing lines. These mice are being analyzed for body weight, motor performance, stride length, pathologies and survival. Our results show that PFN1 transgenic mice appear normal at birth and healthy enough to breed and generate viable offspring. Western blot analysis of total spinal cord protein extract from these three lines demonstrated that mutant human PFN1 protein was expressed at 8.9 folds in H, 2.8 folds in M and 1.1 folds in L expressing lines. The H line mice develop ALS-like phenotypes; i.e. hindlimb tremor, clasping, gait abnormality, low body profile, reduced stride length, gradual weakness and atrophy in muscle of limbs, kyphosis toward later part of the disease, and reduced life-span. They exhibit significant reduction in stride length at symptomatic age (22 ± 0.5 mm vs. 75 ± 1.0 mm in

wildtype controls), this stride length turned to zero at end stage of disease. Immunohistochemical analysis from H line spinal cord sections confirms over-expression of human PFN1 relative to mouse wild-type PFN1. Staining with the astrocyte marker, glial fibrillary acidic protein, also indicate astrocytosis.The average survival is 177 ± 5 days for H line (n=9). The other two lines have subtle phenotypes that progressively slow and are still alive at 400 days, so far. To our knowledge this model is the first to be produced and develop symptoms and signs that resembles ALS. 1. Miller, J. Q.(1963). Lissencephaly in 2 Siblings. Neurology 13:841-850.,2. Wu, C. H. et al.(2012). Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis.Nature 488(7412):499-503.

Disclosures: **S. Yadav:** None. **M. Kiaei:** None.

Poster

696. Motor Neuron Disease and Animal Models

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant 1R01NS077863-01

ANR-2010-BLAN-1429

TARGET ALS

Fondation Thierry Latran "OHEX project"

Title: Synaptic boutons and dendritic vacuolization in adult motoneurons of mSOD1 mouse model of ALS

Authors: N. DELESTRÉE[1], C. MARTINOT[1], M. MANUEL[1], *D. ZYTNICKI[2];
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Abstract: A striking pathological feature observed in mSOD1 mouse models of ALS is the presence of vacuoles in the soma, the axon and also the dendrites of their motoneurons (Dal Canto & Gurney 1994). The mechanism leading to the vacuolization is not known. However, vacuoles might originate through expansion of the mitochondrial intermembrane space (Higgins et al. 2003). We investigated where the vacuoles appear along the dendritic tree, how they evolve

during the time course of the disease and how synaptic boutons are distributed with respect to the dendritic vacuoles. Spinal motoneurons were intracellularly labelled with neurobiotin in anaesthetized mice. After intracardiac perfusion of the mouse, spinal sections were processed for immunostaining of excitatory (VGLUT1 and VGLUT2) and inhibitory (VGAT) boutons. Z-stacks were analyzed using Neurolucida software. We show that both WT and SOD1-G93A mice display swellings along their dendrites (same density: WT 67±28 vs. mSOD1 65±21 swellings/mm), but that vacuolization appears in the dendritic swellings of mSOD1 motoneurons. This vacuolization process has already started by P40 and precedes the denervation of muscle fibers. Afterward, dendritic vacuoles dramatically grow throughout the disease to reach diameters as large as 15 micrometers at P110. The average densities of excitatory (VGLUT1 and VGLUT2) and inhibitory (VGAT) boutons that contact the dendritic tree are unchanged at P40-50 in mSOD1 mice compared to WT mice (VGAT: WT 0.54±0.09 vs. SOD1 0.45±0.08 boutons/µm; VGLUT1: WT 0.06±0.04 vs. mSOD1 0.07±0.02 boutons/µm; VGLUT2: WT 0.42±0.10 vs. mSOD1 0.49±0.07 boutons/µm). Unexpectedly, we found that VGLUT2 and VGAT boutons tend to cluster on swellings in both WT and mSOD1 dendrites creating hotspots of extensive ion influx and high metabolic activity. Our data suggest that there might be a causal link between synaptic activity and dendritic vacuolization in spinal motoneurons of ALS mice, reinforcing the hypothesis that synaptic excitotoxicity can lead to mitochondrial damages and ultimately to degeneration.

Disclosures: **N. Delestrée:** None. **C. Martinot:** None. **M. Manuel:** None. **D. Zytnicki:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.01/G12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: RO3NS074286

Title: Modelling Aggregation

Authors: *T. M. JEITNER;
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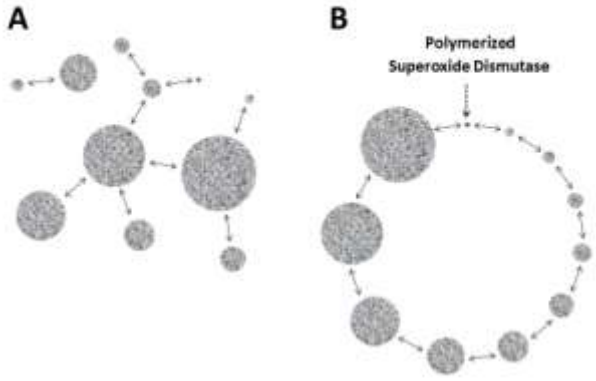
Abstract: All degenerative diseases of the central nervous system exhibit an excess of aggregated material. These assemblages are known by a variety of names depending on the

disease: tangles and plaques in Alzheimer Disease and inclusions in either Amyotrophic Lateral Sclerosis or Huntington Disease. Electron micrographs reveal that aggregates consist mainly of spherical particles bound together. The particles represent polymers, which in most cases, are composed of proteins such as superoxide dismutase in Amyotrophic Lateral Sclerosis or huntingtin in Huntington Disease. In contrast, neuromelanin is an aggregate consisting mainly of polymers of oxidized dopamine. The forces between the particles within aggregates have not been elucidated. These forces are potential target for therapeutics designed to disrupt aggregates in neurodegenerative disorders. Flow cytometers measure the light scattering properties of particles or cells within a population. This technology was used to measure the *in vitro* aggregation of mutant and wild-type superoxide dismutase as well as a pseudo-melanin. These studies allowed the development of a model for aggregation which posits that particles to form aggregates of varying size which achieve equilibrium through the rapid exchange of constituent particles (Fig. 1A). The idealized path of a particle through a population of aggregates of increasing diameters is shown in Fig. 1B. The fact that aggregates rapidly exchange particles indicates that the points-of-contact for the particles are accessible albeit for brief intervals, and therefore, amenable to antagonism. Studies with superoxide dismutase exposed to proteases demonstrated the remarkable observation that these points-of-contacts are encoded in the primary sequence of this protein. These findings imply that compounds that disrupt the binding of particles at the points-of-contact will disassemble aggregates. Since the bonds that stabilize aggregates are weak and form slowly, these represent a viable target for the disruption of aggregates in neurodegenerative disorders.

Figure 1: Model for aggregation



Disclosures: **T.M. Jeitner:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.02/H1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Autophagy contributes to Lewy body formation in a human cell model of Parkinson's disease

Authors: *P. A. TRIMMER[1], E. CRONIN-FURMAN[3], K. BERGQUIST[2];
[1]Parkinson's and Movement Disorders Ctr. and Anat. and Neurobio., [2]Parkinson's and Movement Disorders Ctr., Virginia Commonwealth Univ., Richmond, VA; [3]Neurosci. Grad. Program, Univ. of Virginia, Charlottesville, VA

Abstract: Lewy bodies (LB) are abnormal aggregates found in neurons of the substantia nigra and other regions of the brain and nervous system in Parkinson's disease (PD). Proteins involved in macroautophagy (autophagy) are found in LB, implicating a role for this protein degradation pathway in LB formation. Autophagosomes enclose and degrade damaged and dysfunctional cytosolic proteins (e.g. alpha synuclein) and organelles (e.g. defective mitochondria). Lysosomes fuse with autophagosomes to deliver enzymes that degrade autophagosome contents. Proteins such as the autophagosome membrane protein LC3 and the lysosome membrane protein LAMP2A have been detected in LB. Relatively little is known about LB formation, in part due to a lack of models that generate LB-like intracellular aggregates. We explored the role of autophagy in LB formation using the cybrid model of PD. Cybrid cell lines result from the fusion of platelets from individual sporadic PD or control donors with mtDNA-free (Rho0) human neuroblastoma cells (SH-SY5Y). The PD and control cybrid cell lines generated express mitochondrial DNA (mtDNA) unique to each donor. This model is unique because PD cybrid cells spontaneously produce abnormal intracellular aggregates (cybrid LB, CLB) that replicate the composition and structure of LB found in PD brain. Markers of autophagy (LC3, LAMP2A) and mitochondria (MitoTracker) were visualized in cybrid cells after treatment with vehicle or the vacuolar ATPase inhibitor bafilomycin A1 (BAF, 25nM for 24 hours) to inhibit the fusion of lysosomes with autophagosomes. We used electron microscopy (EM) and immunocytochemistry to visualize autophagosome distribution and mitochondrial morphology in BAF or vehicle-treated PD and control cybrid cells. In addition, viral LC3-GFP vector-expression and live confocal microscopy was used to image LC3 trafficking to the CLB. We found that LC3 and LAMP2A co-localized with classic markers of LB in CLB (ubiquitin, Congo red). LC3-GFP was trafficked to CLB in the presence and absence of BAF, suggesting that autophagosomes are involved in the addition of contents to CLB. We also observed the addition of early autophagosomes to CLB in the presence of BAF by EM. Some BAF-treated cybrid cells exhibited fragmented mitochondria suggesting that inhibition of autophagy permits deteriorating mitochondria to accumulate. Finally, autophagosomes were trafficked to CLB, independent of

lysosomal function and autophagosome-lysosome fusion. This suggests that autophagy plays a key role in LB formation and/or maintenance. Future studies will focus on manipulation of autophagy to modify LB formation.

Disclosures: **P.A. Trimmer:** A. Employment/Salary (full or part-time):; VIrginia Commonwealth University. **E. Cronin-Furman:** A. Employment/Salary (full or part-time):; University of Virginia. **K. Bergquist:** A. Employment/Salary (full or part-time):; VIrginia Commonwealth University.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.03/H2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Parkinson's and Movement Disorders Center at Virginia Commonwealth University

Title: Differentiation of human neural stem cells to a motor neuron phenotype stimulates mitochondrial biogenesis

Authors: ***L. O'BRIEN**[1,2], J. P. BENNETT, Jr.[1,2];
[1]Virginia Commonwealth Univ., Richmond, VA; [2]VCU Parkinson's and Movement Disorders Ctr., Richmond, VA

Abstract: Mitochondrial biogenesis (mitobiogenesis) is the process by which cells increase their mitochondrial mass and includes transcription of genes encoded by both the mitochondrial and nuclear genomes. Mitochondrial function declines in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). ALS is characterized by death of motor neurons in the brain and spinal cord. Despite extensive study, the mechanisms underlying the pathogenesis of sporadic ALS are unknown. However, decreased expression of mitobiogenesis genes, decreased activity of electron transport chain subunits and deletions in mitochondrial DNA (mtDNA) have been found in postmortem tissues of sporadic ALS patients. The use of human pluripotent stem cells (hPSCs) has allowed us to study previously inaccessible cells like neurons. Previous research suggests a switch from glycolysis to mitochondrial oxidative phosphorylation during the spontaneous differentiation of hPSCs into cells of all three germ layers. This process is reversed during the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). However, the mechanisms underlying this switch remain unclear.

We hypothesized that mitobiogenesis is increased during motor neuron differentiation. To test this, we used retinoic acid (RA) and purmorphamine (PM), a sonic hedgehog agonist, to differentiate commercially available human neural stem cells (hNSCs) into motor neurons in low (5%) oxygen conditions. After exposure to RA and PM hNSCs increased mRNA and protein expression of genes involved in motor neuron development. Electrophysiological recordings including whole cell voltage and current clamp confirmed the maturation of neurons. These cells also increased expression of peroxisome proliferator-activated receptor gamma, co-activator 1-α (PGC-1α), an upstream regulator of transcription factors involved in mitobiogenesis, as well as its downstream targets. This was correlated with a 2.5 fold increase in mtDNA copy number and increased protein expression of electron transport chain subunits, suggesting an increase in mitochondrial mass with differentiation into motor neurons. Our findings suggest that mitochondrial biogenesis is increased during differentiation of hNSCs into a motor neuron-like phenotype. Future studies will focus on iPSCs reprogrammed from sporadic ALS patient cells to see if they have the same mitochondrial deficits seen in post mortem tissues.

Disclosures: L. O'Brien: None. **J.P. Bennett:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.04/H3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ALS Worldwide Foundation

Parkinson's Movement and Disorders Center (PMDC), VCU

Title: Effects of a microneurotrophin on gene expression in an *in vitro* human motor neuron model

Authors: *D. G. BROHAWN[1], L. O' BRIEN[1], A. GRAVANIS[2], J. BENNETT, JR.[1];
[1]Virginia Commonwealth Univ., Richmond, VA; [2]Univ. of Crete, Heraklion, Greece

Abstract: Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disorder involving death of motor neurons in the cerebral cortex, brain stem, and spinal cord. Their loss leads to progressive muscle weakness and atrophy with respiratory failure being the leading cause of death an average of 3-5 years after diagnosis. Previous ALS studies have relied on animal

models with disappointing results, as treatment options remain limited and only extend lifespan by several months. Dr. Achilleas Gravanis, Professor of Pharmacology at the University of Crete, provided us an active and an inactive analogue of a microneurotrophin he recently found to have neuroprotective effects. We will use the Illumina Human HT-12 v4 Expression BeadChip assay to run a blind experiment comparing gene expression differences in a human motor neuron cell line treated with the active analogue and inactive analogue. We induced motor neurons by treating Gibco® human neural stem cells (hNSCs; Invitrogen), which are derived from the NIH approved undifferentiated human embryonic stem H9 cell line, with neurobasal medium containing 2% B-27 serum-free supplement, 2 mM GlutaMax-I supplement, and 0.1 uM retinoic acid (RA) for 7 days followed by 7-21 days of 0.1 uM RA plus 0.5 uM purmorphamine (PM). Cells were grown to confluency in four T75 flasks and treated with 100 nM of the active analogue, 100 nM of the inactive analogue, 100 ng/mL NGF (nerve growth factor), or empty vehicle for 24 hours. All treatment compounds were solubilized equally in DMSO prior to administration. 500 ng of RNA was harvested from each treatment group and input into the microarray assay. We repeated this experiment three times to produce replicates for analysis. Data will be analyzed using a combination of Illumina GenomeStudio and the open source software Limma to identify differentially expressed genes and fold changes in each group. Differentially expressed genes identified in cells treated with the active analogue will be input into Ingenuity's Pathway Analysis to identify responsive molecular pathways. Results presented will include a summary of fold changes for all differentially expressed genes in the treatment condition, a schematic of the interconnected biological pathways affected by the active analogue, and interpretation of these effects within the context of ALS literature. Ultimately, this study could provide insight into what underlies this drug's previously observed neuroprotective effect.

Disclosures: D.G. Brohawn: None. **L. O' Brien:** None. **A. Gravanis:** None. **J. Bennett, Jr.:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.05/H4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Mitochondrial oxidative phosphorylation transcriptome alterations in human amyotrophic lateral sclerosis spinal cord and blood

Authors: *A. C. LADD, P. M. KEENEY, J. P. BENNETT;
Virginia Commonwealth Univ., Richmond, VA

Abstract: Origins of onset and progression of motor neurodegeneration in amyotrophic lateral sclerosis (ALS) are not clearly known but may include impairment of mitochondrial bioenergetics. We used quantitative PCR approaches to analyze the mitochondrial oxidative phosphorylation (OXPHOS) transcriptomes in human spinal cord tissue and peripheral blood mononuclear cells (PBMC) from persons with sporadic ALS compared to those without neurological disease. We queried both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) transcripts, both of which translate to provide the necessary protein components of the OXPHOS system. Gene expression measurements from post-mortem, whole spinal cord tissue sections, comprised mainly of supportive glial cells, were compared to that of patient-matched individual motor neurons collected via laser capture microdissection (LCM). PBMC samples were collected from an independent set of patients living at varying, but earlier stages of disease compared to the spinal cord specimens. Results showed the most significant differences were found in mtDNA-encoded respiratory gene expression, which was significantly decreased in both ALS spinal cord and freshly isolated PBMC from ALS patients. nDNA encoded OXPHOS genes showed heterogeneously and mostly decreased expression in ALS spinal cord tissue. In contrast, ALS PBMC showed no significant change in expression of nDNA OXPHOS genes compared to controls. Expression changes in isolated motor neurons are ongoing. Genes related to mitochondrial biogenesis (PGC-1alpha, TFAM, ERRalpha, NRF1, NRF2 and POLgamma) were queried with inconclusive results in both spinal cord and PBMC. Here, we demonstrate there is a systemic decrease in mtDNA gene expression in ALS central and peripheral tissues that supports pursuit of bioenergetic-enhancing therapies. We also identified a combined nDNA and mtDNA gene set (n=26), down regulated in spinal cord tissue, that may be useful as a biomarker in development of cell-based ALS models.

Disclosures: A.C. Ladd: None. **P.M. Keeney:** None. **J.P. Bennett:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: JPSP KAKENHI Grant 23110571

JPSP KAKENHI Grant 23111004

JPSP KAKENHI Grant 25670099

Title: Selective autophagy of lysosomes with ceroid-lipofuscin in neurons deficient in cathepsin D

Authors: T. NANAO[1], M. KOIKE[2], J. YAMAGUCHI[1], C. WHITEHOUSE[3], T. ISHII[4], M. SHIBATA[5], *Y. UCHIYAMA[1];
[1]Cell. and Mol. Neuropathology, [2]Cell biology and Neurosci., Juntendo Univ. Grad Sch. of Med., Tokyo, Japan; [3]Dept of Med. Mol Genet, King's Coll, London, United Kingdom; [4]Sch. of Med., Univ. of Tsukuba, Ibaraki, Japan; [5]Div. of Gross Anat. and Morphogenesis, Niigata Univ. Sch. Med. & Dent. Sci., Niigata, Japan

Abstract: p62 and NBR1, adaptor proteins for selective autophagy, have binding regions with ubiquitin and LC3 on the isolation membrane of autophagosomes (AP). We have previously shown that mice deficient in lysosomal cathepsin D (CD) exhibit a new form of lysosomal accumulation disease with a phenotype resembling neuronal ceroid lipofuscinosis (NCL). Electron microscopic observations revealed accumulation of granular osmiophilic deposits (GRODs) and AP, morphological hallmarks of NCL, in the perikarya of neurons deficient in CD. Since GRODs were frequently found within AP that were localized in the perikarya of CD-deficient neurons, we speculated that enwrapment of GRODs into AP is due to selective autophagy. By immunostaining at the light and electron microscopic levels, ubiquitin was found to be colocalized with LC3, p62, and NBR1 in CD-deficient neurons, while gold particles for ubiquitin was detected on the membrane of GRODs together with those for p62 or NBR1. In CD-deficient neurons, GRODs, p62 and NBR1 were found only in somatodendritic portions but not in axons and their terminals. When triple knockout mice of CD, p62 and NBR1 in mouse brains were produced and analyzed, GRODs were not detected in AP of the neurons. In primary cultured neurons obtained from mouse embryonic cerebral cortex at E16, Lysotracker red-positive acidic compartments were largely localized in cell bodies and dendrites at 10 days after the start of cultures (DIV), although such positive vesicles were abundantly present in axons and filopodia at 3 DIV. Moreover, not only endogenous p62 and NBR1 but also GFP-tagged p62 and NBR1 were detected only in cell bodies and dendrites but not in the distal part of axons beyond the ankyrin G-positive initial segment. Furthermore, by producing mutant molecules of p62 and NBR1, we found that the limited localization of p62 and NBR1 in neurons was required to form homo- or hetero-oligomers with each other in the neurons. These results indicate that AP is non-selectively formed in the axons and axon terminals, while it is retrogradely sent back to the cell bodies of neurons where it receives lysosomal enzymes and become autolysosomes.

Disclosures: T. Nanao: None. **Y. Uchiyama:** None. **M. Koike:** None. **J. Yamaguchi:** None. **C. Whitehouse:** None. **T. Ishii:** None. **M. Shibata:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.07/H6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH/NIA K01AG039386

Schott Foundation

Title: Cdk5/p25 - a novel target for prion disease

Authors: *M. AHN, A. OEHLER, S. DEARMOND;
Dept. of Pathology, UCSF, San Francisco, CA

Abstract: Prion disease is one of the most devastating neurodegenerative diseases. Unlike other neurodegenerative diseases, it is caused by a single pathogen, pathogenic prion protein (PrPSc), which is converted from the normal cellular prion protein (PrPC). However, physiological roles of PrPC and signaling pathways involved in prion disease are still poorly understood. Here, I report that cyclin-dependent kinase 5 (Cdk5) plays an important role in pathological processes involved in prion disease. We observed that Cdk5 directly interacts with PrPSc and the level of p25, a cleaved form of p35 (Cdk5 activator), which has a longer half-life leading to hyperactivation of Cdk5, increases as prion disease progresses *in vivo*. Inhibition of Cdk5 with its inhibitor, roscovitine, or Cdk5-siRNA reduced the level of PrPSc *in vitro*. Lastly, an inhibitory peptide blocking formation of the Cdk5/p25 complex prevented PrPSc formation/propagation and dendritic degeneration in RML-infected brain aggregates. These data support that Cdk5 signaling pathways play an important role in prion disease and interruption of the Cdk5/p25 complex may be a novel strategy to treat prion disease.

Disclosures: M. Ahn: None. **A. Oehler:** None. **S. DeArmond:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.08/H7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Pathological roles of VEGF/SphK pathway in Niemann-Pick Type C neurons

Authors: *H. JIN[1], H. LEE[1], J. LEE[2,3], M. PARK[2,3], J.-S. BAE[2,3];
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Abstract: Sphingosine is a major storage compound in Niemann-Pick type C disease (NP-C), although the pathological role(s) of this accumulation have not been fully characterized. Here we show that sphingosine kinase (SphK) activity is reduced in NP-C patient fibroblasts and NP-C mouse Purkinje neurons (PNs) due to defective vascular endothelial growth factor (VEGF) levels. Sphingosine accumulation by inactivation of VEGF/SphK pathway led to PNs loss via inhibition of autophagosome-lysosome fusion in NP-C mice. VEGF released from bone marrow mesenchymal stem cells (BM-MSCs) also activated SphK by binding to VEGFR2, resulting in decreased sphingosine storage as well as improved PN survival and clinical outcomes in NP-C cells and mice. Similar effects were noted after genetic and pharmacologic replenishment of VEGF in NP-C mice by correction of sphingosine-mediated autophagic dysfunction. Further, induced pluripotent stem cells (iPSC)-derived human NP-C neurons were generated for the first time and the abnormalities caused by VEGF/SphK inactivity in these cells were corrected by replenishment of pure VEGF or VEGF released from BM-MSCs. Overall, these results reveal a novel pathogenic mechanism in NP-C neurons where defective SphK activity is due to impaired VEGF level, and suggests that enhancing SphK activity is a potential new therapeutic intervention for this disorder. This work was supported by the Bio & Medical Technology Development Program (2012M3A9C6050107; 2012M3A9C6049913) of the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT & Future Planning, Republic of Korea.

Disclosures: **H. Jin:** None. **H. Lee:** None. **J. Bae:** None. **J. Lee:** None. **M. Park:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.09/H8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Grant-in-Aid 23591241

Title: Impaired axon-glia interactions cause axonal degeneration and cell death of neurons in the cerebellum of Caspr-deficient shambling mice with progressive neurological phenotypes in aging

Authors: *Y. TAKAGISHI, H. OOHORI, H. MIZOGUCHI;
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Abstract: The paranodes flanking nodes of Ranvier in myelinated nerves are a site of axon-glia interaction, which is crucial fro organization and maintenance of myelinated nerves. Disrupted formation of the papanode has been shown in neurological mouse mutants and human neurodegenerative disease. Shambling (*shm)* is a neurological mouse mutant and lacks the paranodal junction as a result of a mutated gene that encodes a paranodal membrane protein Caspr. The *shm* mice develop ataxic gait at early postnatal days and thereafter display progressively severer motor disabilities, hind-limb paralysis and stiffed bodies. In light of advanced motor disabilities seen in *shm* mice, we performed behavior and morphological analyses to assess if the cerebellum was affected in development and aging. The locomotor activity measured by the home cage activity test and the open field test revealed that aged (over 1 year old) *shm* mice were significantly severer affected in the motor performance compared to adult (3 months old) mice. Histological examination demonstrated that cerebellar formation such as foliation, layer formation and cellular organization was normal in *shm* mice at P13-17 and 3 months old. Immunohistochemistry (IHC) for calbindin D-28K and IP3 receptors, however, displayed that some Purkinje cell (PC) axons had local swellings at 3 months old. The PC axonal swellings were more pronounced and some PCs were lost at 6 months old. An extensive PC loss was found in the broad region of the cerebellum in aged *shm* mice. IHC for SMI31 (phosphorylated neurofilament, p-NF-H) and SMI32 (non phosphorylated NF-H) showed that PC axonal swellings contained aggregation of NF-H. Although both SMI31 and SMI32 were reduced in total from the cortex and medulla of aged mice, the SMI32 was strongly distributed in some PC somata and the pinceau formation, indicating that the NF-H were highly aggregated in these structures. EM analysis showed that PC axonal swellings contained ER stacks, clustered mitochondria and autophagy-like organelles. Altered organelles were also present in aged PC somata. Taken together, the present finding demonstrated that cytoplasmic and cytoskeletal disorganization of PC axons started from the adult age and progressively resulted in the degeneration of PC soma and cell death. These abnormalities are associated with progressive neurological phenotypes of *shm* mice in aging.

Disclosures: **Y. Takagishi:** None. **H. Oohori:** None. **H. Mizoguchi:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.10/H9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ARAMISE (French MSA patients association)

Title: Region-specific alterations of matrix metalloproteinase activity in multiple system atrophy

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Abstract: Multiple system atrophy (MSA) is a neurodegenerative disorder characterized by a combination of autonomic dysfunction, cerebellar ataxia and parkinsonism. The hallmark of MSA is the accumulation of α-synuclein (α-syn) protein aggregates in oligodendrocytes forming glial cytoplasmic inclusions (GCIs). Considered as a primary oligodendrogliopathy, MSA is characterized by myelin deterioration accompanied by secondary neuronal loss and neuroinflammation as well as blood brain barrier (BBB) dysfunction. MMPs are zinc-dependent endopeptidases involved in the remodeling of the extracellular matrix, demyelination and BBB permeability. Several lines of evidence indicate a role for these enzymes in various pathological processes including stroke, multiple sclerosis, Parkinson's and Alzheimer's disease. Their potential involvement in multiple system atrophy is currently unknown. In this study, we aim to assess the potential role of MMPs in MSA pathophysiology by looking at MMP-2 and MMP-9 by in MSA postmortem brain tissue. Zymography revealed increased MMP-2 activity in the putamen of MSA patients relative to controls (+ 27%, $p<0.05$) but not in the frontal cortex. No significant difference was found for MMP-9. Immunohistochemistry against MMP-2 revealed an increased number of glial cells positive for MMP-2 in the putamen. Double immunofluorescence revealed that MMP-2 colocalized with α-syn in GCIs. These results indicate that the activity of MMP-2 is increased in MSA specifically in a brain region that display severe neurodegeneration (putamen) but remains normal in an area relatively spared by the disease process (frontal cortex). Elevated MMP-2 activity in the putamen in MSA may thus contribute to the disease process by promoting blood brain barrier dysfunction and/or myelin degradation.

Disclosures: F. Bassil: None. **A. Monvoisin:** None. **M. Canron:** None. **A. Vital:** None. **W. Meissner:** None. **F. Tison:** None. **P. Fernagut:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.11/H10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Characterization of the insulin/IGF-1 signaling pathway in multiple system atrophy

Authors: M.-H. CANRON[1], F. BASSIL[1], A. VITAL[1,2], E. BEZARD[1,3], P.-O. FERNAGUT[1], *W. MEISSNER[1,3,4];
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Abstract: Multiple system atrophy (MSA) is a sporadic, progressive neurodegenerative disorder characterized by a various combination of parkinsonism, cerebellar ataxia and autonomic dysfunction. The pathological hallmark of this disorder is the accumulation of α-synuclein aggregates in the cytoplasm of oligodendrocytes. Recent studies indicate that insulin like growth factor-1 (IGF-1) serum levels are increased in MSA patients and that IGF-1 brain tissue levels are reduced in a transgenic MSA mouse model. Alterations of the insulin/IGF-1 signaling pathway have been observed in other neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease and targeting it in preclinical studies has shown beneficial effects on behavioral outcomes and surrogate markers of neurodegeneration. In this study we assess insulin/IGF-1 signaling in human postmortem brain tissue of MSA patients and healthy controls. Immunohistochemistry was used to measure the expression of insulin receptors, IGF-1R, glucagon like peptide-1 receptors (GLP-1R), activators of the insulin/IGF-1 signaling pathway along with the downstream effector FOXO-1 in the putamen and frontal cortex. The number of IGF-1R and FOXO-1 positive cells is significantly increased in the putamen and cortex of MSA patients. IGF-1R, GLP-1R and insulin receptor positive cells are also significantly increased in the cortex but not in the putamen (albeit a trend was noted for increased expression). These results indicate that the insulin/IGF-1 pathway is altered in MSA in brain regions that display severe (putamen) or modest (frontal cortex) neurodegeneration. Further investigation will allow determining if increased expression is secondary to massive neurodegeneration in MSA or contributes to the disease process by altering glial function.

Disclosures: **M. Canron:** None. **F. Bassil:** None. **W. Meissner:** A. Employment/Salary (full or part-time):; University Bordeaux. 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.; Michael J Fox Foundation, the University Hospital Bordeaux, the French Health Ministry, the European Community and PSP-France. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); Teaching honoraria and travel grants from Affiris, Lundbeck, Novartis, TEVA and UCB. **E. Bezard:** None. **A. Vital:** None. **P. Fernagut:** None.

Poster

697. Other Neurodegenerative Disorders I

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: FAPESP Grant 2010/51344-3

CAPES

CNPq

Title: Dystrophin deficient-mice present increased concentrations of gamma-aminobutyric acid (GABA) and excitatory amino acids in the cerebellum

Authors: ***M. I. B. FRANGIOTTI**[1], J. D. P. DA SILVA[1], E. F. DE CASTRO NETO[2], P. V. V. SOUSA[2], M. G. NAFFAH-MAZZACORATTI[3], C. SOUCCAR[1];
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Abstract: Duchenne muscular dystrophy (DMD) is a progressive and irreversible muscle degeneration caused by mutations in the dystrophin gene that result in a nonfunctional or lack of the protein. On third of patients with DMD also present cognitive deficits and neuropsychiatric disorders. Dystrophin is normally expressed at the post-synaptic densities of the neuronal synapses in the cortex, hippocampus and cerebellum. The lack of dystrophin (427 kDa) in the dystrophic (mdx) mouse has been associated with a decrease in the size and number of clusters of GABAA receptors (GABAAR) α1 and α2 subunits in the cerebellum and hippocampus. In

addition, the frequency of miniature inhibitory postsynaptic currents (mIPSCs) was decreased in cerebellar Purkinje cells and increased in hippocampal CA1 pyramidal cells of mdx mice (Pilgram et al., Mol. Neurobiol.2010, 41:1). These observations have suggested a role of dystrophin in synaptic function in the CNS. The aim of this work was to evaluate the influence of dystrophin on the concentrations of GABA, aspartate (Asp), glutamate (Glu) and glutamine (Gln) in homogenates of the cerebral cortex, hippocampus and cerebellum of 4-month-old control and mdx mice. The concentrations of the amino acids were determined using high performance liquid chromatography (HPLC) with fluorometric detection. All experimental protocols were approved by the institutional Animal Investigation Ethics Committee (Protocol CEUA Nº 9049101316). The results showed that dystrophin deficiency resulted in significant increase in the concentrations of GABA (44%), Asp (45%), Glu (35%) and Gln (44%) in the cerebellum of mdx mice, compared to control values. A significant decrease in the concentration of GABA (14%) was detected in cortical homogenates of mdx mice compared to control values. In the hippocampus, no significant difference in the concentration of either amino acid was detected between control and mdx mice. The results indicate that the cerebellum was the most affected by dystrophin deficiency, presenting an increased content of GABA, Asp, Glu and Gln in mdx mice. These data may be related to the reported reduction in the number of GABAAR clusters and the frequency of spontaneous mIPSCs in the cerebellum of mdx mice, and may contribute to the cognitive impairments described in the murine model and patients with DMD.

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Poster

697. Other Neurodegenerative Disorders I

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Support: NIH Grant R01AG038791

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Tau Research Consortium

Title: Longitudinal assessment of oculomotor metrics in patients with progressive supranuclear palsy (PSP)

Authors: *H. W. HEUER, L. VOLTARELLI, B. YANG, A. L. BOXER;
Univ. California San Francisco, SAN FRANCISCO, CA

Abstract: Progressive supranuclear palsy (PSP) is a neurodegenerative disease characterized by impaired vertical gaze, gait disturbances and balance difficulties, axial rigidity, and subtle behavioral and cognitive symptoms. Oculomotor impairment is one of the primary clinical indicators distinguishing PSP from other motor and cognitive disorders; however, the time course of saccadic changes has not been fully characterized. To learn more about the progression of symptoms in PSP, saccadic eye movements were measured longitudinally in patients with diagnoses of PSP and in healthy elder controls. Using an infrared eye-tracker, we examined the latency, amplitude, and velocity of horizontal and vertical saccades to visual targets at five and ten degrees eccentricity. Measurements were taken at baseline and approximately six months apart (range 5-8mos). At baseline, patients with PSP exhibited lower saccade amplitudes and velocities than healthy controls, as well as longer initiation times. As a group, PSP patients showed significant increases in latency for saccades to vertical targets at the second time point (TP2) relative to baseline; the latencies for individual subjects were also longer at TP2. Significant decreases in initial gain were also seen for less eccentric vertical targets. Additionally, there was a trend towards decreased velocity for both horizontal and vertical eye movements. These findings suggest that changes in oculomotor metrics may serve as an indicator for disease progression and provide a quantitative assessment tool for evaluating the effectiveness of targeted therapeutic agents.

Disclosures: H.W. Heuer: None. **L. Voltarelli:** None. **B. Yang:** None. **A.L. Boxer:** 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.; BMS, C2N, Eli Lilly, Genentech, Janssen, Pfizer, TauRX. F. Consulting Fees (e.g., advisory boards); Acetylon, Archer, Ipieran, Isis, Neurophage. Other; CBD Solutions, Alzheimer's Association, Bluefield Project to Cure FTD.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: FAPESP Grant 2010/51344-3

CAPES

CNPq

Title: [3H]-GABA release evoked by nicotinic stimulation is reduced in cerebellar synaptosomes of dystrophic (mdx) mice

Authors: *J. D. DA SILVA[1], M. I. B. FRANGIOTTI[1], F. M. NOGUEIRA[1], R. S. STILHANO[2], R. M. DA SILVA[3], G. M. KO[3], S. W. HAN[2], C. SOUCCAR[1];
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Abstract: Cognitive impairments have been described in 30% of patients with Duchenne muscular dystrophy (DMD). DMD is a myopathy caused by mutations in the dystrophin gene and lack of the protein expression. Dystrophin is a structural protein that provides stability to the sarcolemma of striated muscle cells, and is expressed at the post-synaptic membrane of neuronal synapses. Dystrophin deficiency in the mdx mouse has been associated with a reduction in the number and size of GABAA receptor (GABAAR) clusters in the hippocampus and cerebellum (Pilgram et al., Mol. Neurobiol. 2010, 41:1). Altered concentrations of α7- and β2-containing nicotinic acetylcholine receptors (nAChRs), and increase of nAChR-evoked [3H]-ACh release have been also reported in the hippocampus of mdx mice (Parames et al., Neuroscience 2014, 269: 173), indicating a role of dystrophin in synaptic function. Considering the importance of nAChRs in regulating the release of various neurotransmitters including GABA in the CNS, in this study we evaluated the influence of dystrophin on the release of [3H]-GABA evoked by nAChRs activation in synaptosomes prepared with brain regions that normally present high concentration of dystrophin, from 4-month-old control and mdx mice. All experimental procedures were approved by the local Animal Investigation Ethical Committee (Protocol N° 1178/10). Crude synaptosomes prepared with the cerebral cortex (Cx), hippocampus (H) and cerebellum (Cb) of littermate control and mdx mice preloaded with [3H]-GABA (40 nM) were superfused with Krebs-bicarbonate solution containing 0.1 μM atropine and 50 μM aminooxyacetic acid, at 37°C. The results showed that [3H]-GABA release evoked by 10 μM nicotine or 9 mM K+ was dependent on the extracellular Ca2+ concentration in control and mdx preparations. Nicotine-induced [3H]-GABA release from cortical and hippocampal synaptosomes did not differ between control and mdx mice. However, nAChR-evoked [3H]-GABA release from cerebellar synaptosomes was decreased by 63% in mdx compared to control values. K+-evoked GABA-release from Cx, Hc and Cb synaptosomes did not differ between control and mdx groups. The observed decrease in nAChR-evoked [3H]-GABA release are consistent with the increased concentration of GABA detected in the cerebellum of mdx mice (see poster by Frangiotti et al.), and may reflect a compensatory mechanism to the reduction in

GABAAR clustering described in the same brain region. The results indicate alterations in GABAergic synaptic function associated with dystrophin deficiency in the cerebellum, which may contribute to the cognitive deficits described in mdx mice and DMD patients.

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Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.15/I2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Paroxysmal non-kinesogenic dyskinesia in dogs associated with a mutation in the glycosylphosphatidylinositol (GPI) anchor synthesis enzyme PIGN responds to acetazolamide therapy

Authors: *D. P. O'BRIEN[1], R. A. PACKER[3], S. A. THOMOVSKY[4], A. L. KOLICHESKI[2], G. S. JOHNSON[2];
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Abstract: Paroxysmal dyskinesias (PD) are movement disorders characterized by normal consciousness during episodes of involuntary movements which can be dystonic, choreaform, or ballistic and can occur during sleep, in response to movement, or spontaneously. The objectives of this study were to characterize a hereditary PD in Soft-coated Wheaten Terriers (SCWT) and identify the mutation responsible for the disease. --Medical records and video recordings were evaluated for 15 SCWTs and 2 F2 SCWT-poodle crosses with PD. Pedigree information suggested an autosomal recessive inheritance. Affected dogs had episodes of dystonia or uncontrollable flexion/extension movements of the limbs that typically began at 8 months to 3 years of age. The episodes would last for a few minutes up to several hours and became more frequent and severe over time. They occurred when the dogs were awake, but no clear trigger was identified. Benzodiazepines, antiepileptic drugs and muscle relaxants failed to affect the signs. Of 5 dogs treated with acetazolamide, 2 had complete resolution of signs, 1 responded transiently and 2 did not respond. When therapy was stopped in 1 dog, signs returned but resolved when therapy was reinstated. Histopathology of two dogs failed to show any pathology. --Analysis of whole genome sequences from 2 affected dogs identified a plausibly causal variant

in *PIGN*. All 10 affected dogs with DNA available were homozygous for the variant allele and all 456 clinically normal SCWTs were either heterozygous or homozygous for the wild type allele. All 387 poodles and 113 randomly selected other breed dogs were homozygous wild type. --*PIGN* controls the addition of phosphoethanolamine to the first mannose in GPI. GPI anchors over 150 proteins to lipid rafts. As might be expected if GPI function were disrupted, mutations in *PIGN* in children are associated with a very severe, lethal phenotype with multiple congenital anomalies, hypotonia, chorea, and seizures. Our data suggest that mutations in PIGN can produce a paroxysmal non-kinesogenic dyskinesia which can respond to carbonic anhydrase inhibition while permitting normal development. Identification of which GPI anchored protein(s) mediate these effects could shed light on the pathogenesis and treatment of movement disorders.

Disclosures: D.P. O'Brien: None. **R.A. Packer:** None. **S.A. Thomovsky:** None. **A.L. Kolicheski:** None. **G.S. Johnson:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.16/I3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Higher specific infectivity of exosomal prions

Authors: *F. PROPERZI, M. LOGOZZI, H. ABDEL HAQ, E. FERRONI, C. FEDERICI, L. LUGINI, T. AZZARITO;
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Abstract: Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders of humans and animals. During the disease, there is an accumulation in brain of an abnormal partially protease-resistant form of the prion protein (PrPSc). This is a misfolded form of the host-encoded protease-sensitive cellular prion protein (PrPc). It is generally believed that, after binding with PrPSc the PrPc converts to the misfolded pathological form, triggering neurotoxicity. Nevertheless recently many solid data have questioned the equivalence of PrPSc to prions, primarily by showing that in some cases prion infection occurs in absence of PrPSc. In addition, to date, it has not been possible to reproduce infectivity by in-vitro approaches, which challenges the protein only content of prions. The nature of the TSE infectious agent is certainly not as simple as it was originally thought and remains at present elusive, delaying the finding of successful diagnostic and therapeutic strategies. To better

understand the nature of prions we used a novel experimental model: large scale preparations of exosomes released by chronically infected cells. Exosomes are nanovesicles of 50-90nm released by the majority of cells and they have been shown to contain prions when released by infected cells in-vitro. As they are a much less complex material compared to brain tissue the finding of cofactors involved in prion infectivity could be greatly facilitated. Our results confirmed that both prion infectivity and PrPSc released by chronically infected cells are mainly associated to nano-vesicles. In addition, an estimation of specific infectivity relative to the PrPSc content showed for the first time that the PrPSc released by cells on exosomes is at least thirty times more infectious than PrPSc retained in cell lysates. This is a crucial finding that clearly confirms that not all PrPSc molecules associate with the same level of prions. Interestingly infectivity levels of Triton-treated exosomes are significantly increased. This results suggest that lipid rafts retain the highest infectious PrPSc species and might have a role in prion propagation.

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Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.17/I4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: ALS-related functional connectivity changes in the frontal and temporal lobe

Authors: K. LOEWE[1,2], J. MACHTS[3], C. STOPPEL[4,1], S. ABDULLA[1,5], K. KOLLEWE[5], S. PETRI[5], R. DENGLER[5], H.-J. HEINZE[1,6], J.-M. HOPF[1,6], S. VIELHABER[1,3], *M. A. SCHOENFELD[1,6,7];
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Abstract: Recent studies posit amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) to be on opposite poles of one clinical continuum. ALS-related changes in extra-motor areas have been observed using structural MRI but not pronounced in the temporal lobe. Reports on functional connectivity in ALS were also inconclusive with respect to the continuum

hypothesis. Here, we investigated resting-state functional connectivity using whole-brain voxel-level graphs to map ALS-related brain network changes at the level of individual voxel pairs. Patients with classical ALS (N = 64) and age-, gender-, and education-matched controls (N = 38) underwent detailed neuropsychological assessment and resting-state fMRI at 3 Tesla. Subject-specific connectivity graphs were constructed by defining gray matter voxels as nodes and establishing weighted edges by estimating internodal functional connectivity in terms of Pearson correlation between the nodes' associated time series. Edge-level *t* statistics were computed across graphs to assess between-group connectivity differences. The resulting graph of statistics was pruned in order to identify voxel pairs exhibiting significant changes (corrected for multiple testing, FDR ≤ 0.2). Patterns of increased functional connectivity in ALS were detected in prefrontal and frontal areas. These could reflect increased regulatory processes that might serve to compensate the fronto-executive deficits frequently encountered in ALS patients. Decreased functional connectivity in patients with ALS was detected in the bilateral precentral and postcentral gyri, fusiform, lingual and the middle occipital gyri. In addition, patterns of decreased temporo-occipital connectivity spread from medial and inferior temporal lobe (including the hippocampus) areas to middle occipital lobe. The observed decreased motor connectivity is consistent with previously reported structural damage of motor-related areas in ALS. Since the patients exhibited only slight neuropsychological deficits, the extent of extra-motor involvement was, however, unexpected. These findings match the patterns of cerebral degeneration typically observed in FTD and thus support the idea that ALS and FTD are part of one clinical continuum.

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Poster

697. Other Neurodegenerative Disorders I

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.18/I5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Merz Pharmaceuticals

Title: Characterization of essential tremor by kinematic assessments in the upper limb

Authors: O. SAMOTUS[1], F. RAHIMI[1], J. LEE[1], M. JACKMAN[2], *M. S. JOG[3,1];
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Abstract: Essential tremor (ET) is one of the most common movement disorders causing functional disability during postural or voluntary movements in the upper extremities. Treatments for upper limb tremor in ET patients are suboptimal and have adverse effects. Botulinum neurotoxin type A (BoNT-A) injections have been unsuccessful due to inability to visually deconstruct complex movements of upper limb tremors for muscle selection. This study demonstrates how kinematic technology deconstructs multi-joint tremor to tailor BoNT-A injections. 18 ET patients were assessed over 64 weeks using kinematic sensors placed on the wrist, elbow and shoulder joints. BoNT-A injections were administered every 4 months with a follow-up visit six weeks following treatment. Kinematic assessments and tremor rating scales were completed at each visit. Patients in a seated position performed standard scripted tasks at rest, posture and in weight holding states. Goniometers captured flexion-extension and radial-ulnar degree of freedoms (DOF) in wrist, flexion-extension DOF in elbow, and flexion-extension and abduction/adduction DOFs in shoulder. Measurements were processed and kinematic results were provided to the physician. Dosing of BoNT-A and muscle injection sites were determined based on the physician's own clinical experience and kinematic data. Kinematic measurements obtained during postural and weight holding states showed total tremor amplitude, tremor severity, was significantly reduced by 83% in the wrist, 56% in the elbow and 52% in the shoulder joints. Similar Unified Parkinson's disease rating scale scores for tremor items were significantly reduced by 60% and function disability measured by the Fahn-Tolosa-Marin rating scale was improved by 24%. Maximal grip strength was reduced by 16% with limited to no loss in arm function. The use of objective kinematic assessment allows physicians to pinpoint tremor sources in each arm joint, increasing efficacy of BoNT-A treatment.

Disclosures: O. Samotus: None. **F. Rahimi:** None. **J. Lee:** None. **M.S. Jog:** 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.; Dr. Jog is an advisor to Merz Pharma as an expert Movement Disorders Neurologist.. **M. Jackman:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.19/I6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Movement disorders after stroke

Authors: *A. CHAHIDI[1,2], M. CHRAA[3], N. KISSANI[3], M. CHRAA[2], N. KISSANI[2]; [1]Sorbonne Univ., BENI MELLAL, Morocco; [2]Basic & Clin. Neurosciences Res. Laboratory, UCAM, Marrakech, Morocco; [3]Neurol. Dept., Mohamed VI Univ. hospital, Marrakech, Morocco

Abstract: Background: Movement disorder following stroke represent an uncommon situation. It has been suggested that the prevalence of this complication varies from 1 to 5 % in different studies. It represents an interesting condition because of the pathophysiological questions it raises. Objective: The goal of this study is to describe the clinical, paraclinical and evolutive features of patients having suffered a movement disorder in the aftermath of an ischemic stroke. Methods: Authors report a retrospective study from January 2004 to December 2013. This study collected 442 stroke cases. Data were collected 442 patient's folders who were hospitalized for ischemic stroke in the Neurology Department, Mohammed VI university hospital in Marrakesh, Morocco. Our department is a third level structure which covers much of southern Morocco. The diagnosis of ischemic stroke was established in base of clinical and CT scan criteria. Only patients who presented with a movement disorder in the aftermath of stroke were included. Results: Within our 442 stroke patients with ischemic stroke, 18 presented a movement disorder. There are 10 man and 8 women. The mean age was 59 years. Patients presented this complication 3 days to 1 year after the acute episode. We had 8 patients who developed a Parkinsonism syndrome, 4 patients had a chorea, 3 others had an isolated tremor and 3 presented a dystonia. Parkinsonism was the later to develop in our patients whereas chorea developed some days after the stroke. CT scan showed a subcortical ischemic stroke impacting the basal ganglia in all cases. Finally, the evolution was marked by a resolution (subsiding of all symptoms in all of the?) of all cases of dystonia and chorea. On the other hand, patients who had parkinsonism were being followed in our department for up to 6 years without any major improvement. Conclusions: The present series report the clinical, paraclinical and the outcomes of patients who presented a movement disorder after an ischemic stroke. Even with the small number of patients in this study, many finding and suggestions may be developed.

Disclosures: A. Chahidi: None. **M. Chraa:** None. **N. Kissani:** None. **M. Chraa:** None. **N. Kissani:** None.

Poster

697. Other Neurodegenerative Disorders I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 697.20/I7

Topic: D.02. Auditory

Support: Autism Tissue Program

Title: Hypoplasia of the human medial superior olive in chromosome 15q duplication syndrome

Authors: *R. J. KULESZA, JR[1], R. LUKOSE[2];
[1]Anat., Lake Erie Col. of Osteo. Med., Erie, PA; [2]Neurol., Hamot Univ. of Pittsburgh Med. Ctr., Erie, PA

Abstract: Autism (AUT) is a neurodevelopmental disorder characterized by social and communicative impairments, sensory abnormalities and restricted repetitive behaviors. Although the etiology of most cases of AUT is idiopathic, a small number of cases can be attributed to genetic causes, such as chromosome 15q duplications (dup15q). However, recent neuropathological investigations provide evidence for distinct patterns of cortical heterotopias and dysplasias in subjects with dup15q. It is well established that individuals with AUT generally have some degree of auditory dysfunction and we have recently demonstrated significant hypoplasia in the superior olivary complex (SOC), a collection of auditory brainstem nuclei, of subjects diagnosed with AUT. Specifically, we found the medial superior olive (MSO) to contain significantly fewer neurons in subjects with AUT. We therefore hypothesize that the MSO in subjects with dup15q will demonstrate a similar reduction in neuronal number. Herein, we describe results from a quantitative morphometric investigation of post-mortem brainstem tissue from normally developing control subjects, subjects with AUT and subjects with dup15q. Our observations in subjects with AUT support our previous reports, such that the MSO contained significantly fewer neurons. However, the remaining MSO neurons were significantly smaller, more round and abnormally oriented in the nucleus. In subjects with dup15q, we also find that the MSO has many fewer neurons and the remaining neurons are smaller and more round. Additionally, we found that the SOC in subjects with dup15q was more likely to contain ectopic neurons. These results suggest that in the brainstem, these neuropathological conditions (AUT and dup15q) may be associated with similar developmental errors. However, based on the higher incidence of ectopic neurons in dup15q, additional pathfinding errors may occur in this condition.

Disclosures: R.J. Kulesza: None. **R. Lukose:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.01/I8

Topic: C.05. Aging

Support: NIA 2R01AG032236

NIA 5P01AG026572

Norris Foundation

ADRC P50AG005142

Title: Neuroserms as mitochondrial regulators: Reversal of ovariectomy-induced bioenergetic dysregulation

Authors: *Z. MAO[1], J. YAO[1], L. ZHAO[2], K. WONG[1], Y. WANG[1], C. CALDWELL[1], R. BRINTON[1];
[1]Pharmacol. and Pharmaceut. Sci., USC, Los Angeles, CA; [2]Sch. of Pharm., Univ. of Kansas, Lawrence, KS

Abstract: We designed and developed selective estrogen receptor modulators to target the membrane estrogen receptors, referred to as NeuroSERMs. NeuroSERM1 (NS1) is a hybrid structure of 17β-estradiol (E2) and vitamin E and was designed to target a plasma membrane site of estrogen action. NeuroSERM2 (NS2) has a hybrid structure of an isoflavone and a mitochondrial co-enzyme and was designed to target estrogen receptor β. In the current study, we investigated the impact of NeuroSERMs on mitochondrial function *in vitro* and *in vivo*. In primary cultured rat hippocampal neurons, both NS1 and NS2 promoted mitochondrial respiration comparable to E2, whereas only NS1 significantly increased basal aerobic glycolysis in primary neurons. In addition, both NS1 and E2 significantly increased the ratio of mitochondrial fusion to fission whereas NS2 had no impact. In primary mixed glia, both E2 and NS1 significantly increased mitochondrial respiration whereas NS2 had minimal effect. *In vivo* analyses of NeuroSERM effect on brain mitochondrial function were conducted in 3-week ovariectomized (OVX) female mice treated 4-days with either NS1 or NS2 or vehicle. Compared to the OVX group, both NS1 and NS2 increased activity of enzymes involved in mitochondrial bioenergetics, including PDH, αKGDH and COX activity to a magnitude comparable to E2 treatment. Both NS1 and NS2 exerted full agonist activity, comparable to E2, and reversed the OVX-induced decrease in the neuronal glucose transporter (Glut 3), and the glial monocarboxylate transporter 1 (MCT1), whereas only NS2 treatment induced an increase in MCT2 expression. Further, both NS1 and E2 treatment suppressed the OVX-induced increase in lipid peroxidation whereas NS2 had no impact on lipid peroxidation. To investigate NeuroSERM

regulation of brain bioenergetics from a systems-level, we conducted mitochondria and mitochondrial energy metabolism PCR arrays. Compared to the Sham group, OVX induced a significant decrease in genes involved in key regulatory pathways of glucose metabolism and mitochondrial biogenesis, including ESRRA1, PPARA, PPARG, PPARGC1A, IGF1/IGF1R and Insulin/INSR. Both NS1, NS2 and E2 treatment up-regulated expression of these key genes and therefore reversed the OVX-induced dysregulation of brain metabolic pathways. Importantly, neither NS1 nor NS2 treatment induced an uterotrophic effect. Collectively, these data indicate that NeuroSERMs potentiate mitochondrial function *in vitro* and reverse the deficits induced by loss of ovarian hormones *in vivo*. These findings provide preclinical and translational support for NeuroSERMs as estrogen alternatives for the brain.

Disclosures: Z. Mao: None. **J. Yao:** None. **L. Zhao:** None. **K. Wong:** None. **Y. Wang:** None. **C. Caldwell:** None. **R. Brinton:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.02/I9

Topic: C.05. Aging

Support: NIA 2R01AG032236 (to RDB)

NIA 5P01AG026572 (to RDB)

Norris Foundation (to RDB)

Title: Allopregnanolone potentiates brain mitochondrial function

Authors: *J. YAO[1], S. CHEN[2], K. C. WONG[2], R. D. BRINTON[2];
[1]Pharmacol. and Pharmaceut. Sci., Univ. of Southern California, Los Angeles, CA; [2]USC, Los Angeles, CA

Abstract: Previously, we demonstrated that the neurosteroid, allopregnanolone (Allo) increased neural stem cells (NSCs) proliferation *in vitro*, neurogenesis *in vivo* and restored cognitive deficits in the triple transgenic Alzheimer's mouse model (3xTgAD). Further, Allo treatment reduced AD pathology and increased markers of white matter generation. To further explore mechanisms underlying the regenerative actions of Allo, we investigated the impact of Allo on brain mitochondrial function *in vitro* and *in vivo*. Both *in vitro* and *in vivo* analyses were

conducted following 24 hours after Allo exposure. Mitochondrial respiration was assessed using the Seahorse metabolic analyzer in cultured embryonic E18 rat NSCs,neurons, and mixed glia and in purified mitochondria derived from whole brain. *In vitro*, Allo significantly increased mitochondrial respiration in a dose-dependent manner in hippocampal NSCs, neurons and mixed glia (primarily astrocytes). Further, Allo increased mitochondrial dynamics as evidenced by increased mitochondrial velocity, increased fusion events over fission products in primary cultured hippocampal neurons. To investigate the impact of Allo on brain mitochondrial *in vivo*, 6m wildtype and 3xTgAD female mice were ovariectomized for 6 weeks and then subcutaneously administered vehicle or Allo (10mg/kg). Brain mitochondria were assessed for mitochondrial respiration and for expression and activity of mitochondrial bioenergetic enzymes. *In vivo*, Allo treatment reversed the OVX-induced decrease in mitochondrial respiration in female wildtype and 3xTgAD mice to restore magnitude of function comparable to Sham controls. On indicators of bioenergetic system integrity, Allo restored activity and expression of key bioenergetic enzymes including PDH and αKGDH while reversing OVX-induced increase in lipid peroxidation. Gene expression analyses indicated that Allo increased IGF1 and PGC1α bioenergetics, increased APOE pathway associated genes and decreased the Presenilin 1 pathway. Gene expression profiles are consistent with *in vivo* findings and support Allo potentiation of brain bioenergetics, increased cholesterol and Aβ clearance and reduction in Aβ generation. Collectively, data indicate that allopregnanolone potentiates mitochondrial function in neural stem cells, astrocytes and neurons. *In vivo* Allo reversed multiple mitochondrial bioenergetic deficits induced by loss of ovarian hormones. These findings contribute to the preclinical data in support of developing Allo as a systems level therapeutic to sustain and enhance mitochondrial function and to promote the regenerative capacity of the brain.

Disclosures: J. Yao: None. **S. Chen:** None. **K.C. Wong:** None. **R.D. Brinton:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.03/I10

Topic: C.05. Aging

Support: NIA P01AG026572

NIA R01AG032236

Title: Mechanistic pathways linking mitochondrial hydrogen peroxide production and white matter degeneration in the aging mammalian female brain

Authors: *L. KLOSINSKI[1], J. YAO[1], S. CHEN[1], Z. MAO[1], E. TRUSHINA[2], S. K. TIWARI-WOODRUFF[3], L. ZHAO[4], R. D. BRINTON[1];
[1]USC, Los Angeles, CA; [2]Mayo Clin., Rochester, MN; [3]Univ. of California Riverside, Riverside, CA; [4]Univ. of Kansas, Lawrence, KS

Abstract: White matter hyperintensities are an early hallmark of Alzheimer's Disease (AD) which ultimately manifest in later stages of the disease as white matter degeneration. We propose a mechanistic link between white matter hyperintensities / degeneration and mitochondrial H2O2 generation which activates the phospholipase A2 (PLA2) and arachidonic acid (AA) pathway to initiate a cascade of white matter degradation in the aged mouse brain. Female mice were sacrificed at 3, 6, 9, 12, and 15 months of age followed by analyses of gene and protein expression, electron microscopy and immunohistochemical analysis, coupled with enzyme activity. The degenerative cascade begins with a statistically significant increase in PLA2 activation at 12 months of age. Consistent with increased PLA2 enzyme activity, myelin degradation associated genes, AA and alkaline ceramidase, were significantly upregulated at 12 months of age. Upregulation of AA epoxygenase ($p=0.004$) is indicative of an increase in AA availability, while an increase in alkaline ceramidase ($p=0.01$) indicates activation of the sphingomyelinase ceramide pathway. While myelin synthesis genes exhibited a pattern of downregulation between 12 and 15 months of age, myelin degradation genes remained upregulated, indicating that white matter degeneration continues after 12 months of age. Immunohistochemical mapping of changes in white matter fluorescence indicated that white matter area increased in the hippocampal fimbria and anterior commissure between 9 and 12 months, which was followed by a precipitous decline between 12 and 15 months of age. Electron microscopy analysis demonstrated that the increase in myelin intensity between 9 and 12 months of age was due to loss of myelin sheath integrity, consistent with the increase in degradation genes and enzyme activity between 9 and 12 months of age. These findings create a mechanistic foundation and temporal trajectory for progression of white matter degeneration in aged brain.

Disclosures: L. Klosinski: None. **J. Yao:** None. **S. Chen:** None. **Z. Mao:** None. **E. Trushina:** None. **S.K. Tiwari-Woodruff:** None. **L. Zhao:** None. **R.D. Brinton:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.04/I11

Topic: C.05. Aging

Support: NIA 5P01AG026572 (to RDB)

Title: Impact of perimenopausal transition on plasma and cortical steroids levels in a female rat model of human perimenopause

Authors: *E. CADENAS[1], F. YIN[1], R. C. MELCANGI[4], D. CARUSO[4], A. CHRISTENSEN[2], C. J. PIKE[2], R. D. BRINTON[1,3];
[1]Sch. of Pharm., [2]Davis Sch. of Gerontology, [3]Keck Sch. of Med., USC, Los Angeles, CA;
[4]Pharmacol. and Biomolecular Sci., Univ. of Milan, Milan, Italy

Abstract: The mission of our Perimenopause in Brain Aging and Alzheimer's Disease Program Project (P3) is to discover the biological transformations that occur in the aging female brain during the perimenopausal transition that can result in phenotypes predictive of risk for development of Alzheimer's disease (AD) pathology. Neuroactive steroids exhibit a variety of physiological effects on the brain by regulating neuroendocrine, reproductive and synaptic functions, and exert protective actions in experimental models of AD. We investigated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) the levels of multiple neuroactive steroids in cortex and plasma of a rodent model of human perimenopause. Female Sprague-Dawley rats at 9-10 mo of age were stratified into groups according to stage of ovarian senescence, which model key aspects of human perimenopause-menopause transition (Stages of Reproductive Aging Workshop, 2011): regular cycles (4-5 d cycles), irregular cycles (5-8 d cycles), and acyclic (>9 d cycles: constant estrus or constant diestrus). For comparison, we also included the following groups of female rats: regular cycles at age 6 mo, regular cycles ovariectomized (OVX) at age 10 mo, irregular cycles OVX at age 10 mo, and acyclic/constant estrus at age 16 mo. For all animals, cortical and plasma neuroactive steroids including 17β-estradiol, pregnenolone, progesterone and its derivatives, dihydroprogesterone (DHP), tetrahydroprogesterone (THP, allopregnanolone) and isopregnanolone, dehydroepiandrosterone (DHEA), testosterone and its derivatives, dihydrotestosterone (DHT), and 5α-androstane-3α,17β-diol (3α-DIOL) were quantified. Our data indicate that the endocrine transition from regular cycle to irregular cycle during female aging results in a major shift in multiple steroids in both plasma and brain with the brain showing greatest change. As expected, the perimenopausal transition is associated with a decline in 17β-estradiol whereas unexpectedly there was a pronounced rise in progesterone during irregular cycling phase. Despite the rise in progesterone there was a sharp decline in its metabolite, allopregnanolone in brain with no change in plasma. Correlational analyses of steroids levels with gene expression are underway to establish the relationship between neuroactive steroids and critical metabolic, regenerative inflammatory and AD-pathology-related pathways in the brain during perimenopausal transition. Together, these

data provide novel insights into the effects of perimenopausal transition on steroidogenesis and the profile of systems biology regulators of female aging in the periphery and the brain.

Disclosures: **E. Cadenas:** None. **F. Yin:** None. **R.C. Melcangi:** None. **D. Caruso:** None. **A. Christensen:** None. **C.J. Pike:** None. **R.D. Brinton:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.05/I12

Topic: C.05. Aging

Support: NIA 5P01AG026572-07

Title: Hormone therapy in early but not late middle-age reduces microglial activation in female 3xTg-AD mice

Authors: ***A. CHRISTENSEN**, C. J. PIKE;
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Abstract: Menopause results in a gradual loss of the ovarian steroid hormones estrogen and progesterone. Depletion of these hormones leaves women at increased risk for several disorders, including cardiovascular disease, cognitive dysfunction, and dementias including Alzheimer's disease (AD). Steroid hormone loss also promotes obesity, which is positively associated with development of AD and numerous other diseases. One strategy to improve health outcomes in postmenopausal women is the use of estrogen-based hormone therapy (HT). Several studies have evaluated the efficacy of HT to reduce disease risk, but the outcomes have been mixed. A leading idea to explain the discrepant clinical observations is the window of opportunity hypothesis, which posits that beneficial effects of estrogens are limited to a period near the onset of menopause (perimenopause) after which estrogen responsiveness is altered resulting in fewer benefits and more health risks. In the current study, we assessed the concept of a 'window of opportunity' for HT in a transgenic mouse model of AD (3xTg-AD). We also considered how obesity may affect interactions between reproductive aging, AD pathology, and HT. We administered HT (continuous estradiol and cyclic progesterone) to early (7-9 months; consistent with human perimenopause) and late (16-17 months; consistent with human late menopause) middle-aged female 3xTg-AD mice that were maintained on either a normal or high-fat diet for four months. HT reduced β-amyloid burden in early middle-aged mice, an effect that was

significantly attenuated in obese mice. In late middle-aged 3xTg-AD mice, HT did not significantly affect β-amyloid burden in either the lean or obese conditions. One mechanism that may contribute to these interactive relationships is inflammation, which is increased by obesity, reduced by ovarian hormones, and contributes to AD and related diseases. Here, we observed that HT administered during early middle age was effective in significantly reducing microglial activation. Late middle-aged mice showed greater levels of microglial activation and no significant effect of HT. In summary, we have shown that HT reduces β-amyloid accumulation and microglial activation in early middle-age but not at older ages. Further, obesity significantly lessens the benefits of HT. These findings suggest that HT may be most effective during human perimenopause in reducing indices of neuroinflammation and AD-related pathology, a conclusion consistent with the window of opportunity hypothesis.

Disclosures: A. Christensen: None. **C.J. Pike:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.06/J1

Topic: C.05. Aging

Support: NIH National Institute on Aging U01AG031115 to RDB.

NIH National Institute on Aging UF1AG046148 to RDB.

Title: Early stage development for metabolic profiling of iPSC-derived neural cells from familial and late-onset Alzheimer's disease cohorts

Authors: *C. M. SOLINSKY[1], J. YAO[2], J. K. ICHIDA[3], R. D. BRINTON[4];
[1]Clin. Pharm. and Pharmaceut. Econ. & Policy, [2]Pharmacol. & Pharmaceut. Sci., [3]Stem Cell Biol. & Regenerative Med., [4]Clin. Pharm. and Pharmaceut. Econ. & Policy, Pharmacol. & Pharmaceut. Sci., USC, Los Angeles, CA

Abstract: Alzheimer’s disease (AD) is a national and global epidemic with complex pathoetiology including compromised brain metabolic activity and decreased regenerative capacity. Allopregnanolone (Allo) is an investigational neuroregenerative therapeutic, which has been demonstrated to promote neural stem cell (NSC) proliferation and neural differentiation. In the current study, we sought to investigate the impact of Allo on mitochondrial function. We first

determined that Allo improves basal and maximal capacity in cultures of healthy rat NSCs by 20% and 60% respectively and in healthy rat neurons by 20% and 35%. To develop a biomarker of regenerative capacity in humans, we investigated the impact of Allo on human NSCs, human induced pluripotent stem cells (iPSCs) and iPS-derived neural cells. An initial proof-of-concept bioenergetic assay was conducted using NSCs derived from a healthy donor, demonstrating project feasibility and allowed for the identification of 40,000 NSCs/well as the optimal cell density for this cell type in future metabolic assays. Currently we are reprograming presenilin-1 mutation containing cells from familial AD patients. Once iPSC lines containing the mutation lineage and their autologous controls are established, they will be differentiated into NSCs, neurons, astrocytes, and oligodendrocytes and their metabolic phenotype characterized. Additionally, we will reprogram, differentiate, and characterize iPSC lines derived from late-onset AD donors (Coriell-http://ccr.coriell.org). We will then evaluate the efficacy and mitochondrial mechanisms of Allo in rescuing the compromised phenotypes. Outcomes of these analyses will form the foundation for establishing a biomarker of regenerative capacity phenotype, to enrich clinical trials of therapeutics targeting the endogenous regenerative capacity of the brain in persons with AD, regardless of etiology, to determine and monitor responses to therapeutics.

Disclosures: **C.M. Solinsky:** None. **J. Yao:** None. **J.K. Ichida:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents pending on allopregnanolone as a therapeutic for mild cognitive impairment and Alzheimer's disease..

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.07/J2

Topic: C.05. Aging

Support: F31AG044997

R01AG032236

P01AG026572

R01AG024154

R01AG033288

Title: Development of biomarker profiles for early detection of women with an at-Alzheimer's disease risk phenotype

Authors: *J. R. RETTBERG[1], H. DANG[2], H. HODIS[3], R. D. BRINTON[4], W. MACK[2]; [1]Neurosci., Univ. of Southern California, Los Angeles, CA; [2]Preventive Med., [3]Keck Sch. of Med., [4]Pharmacol. and Pharmaceut. Sci., USC, Los Angeles, CA

Abstract: The prodromal phase of Alzheimer's disease begins decades prior to clinically detectable dementia; thus, identification of early biomarkers is critical to identifying at-risk populations. Metabolic changes in the brain are among the earliest features of the Alzheimer's pathological cascade. Estrogen positively regulates the bioenergetic system of the brain from glucose uptake to aerobic glycolysis, mitochondrial function and ATP generation. Estrogen also regulates the peripheral metabolic profile, and peripheral changes in metabolic homeostasis are coincident with metabolic changes occurring in the brain (Rettberg et al. 2014. Front Neuroendocrinol.). Based on this foundation, we proposed that loss of ovarian hormones at menopause could initiate a bioenergetic and metabolic crisis that could lead to a metabolic phenotype consistent with increased risk for AD. To address this hypothesis, we conducted an unbiased principal components analysis followed by k-means clustering of clinical data and bioenergetic indicators derived from plasma from women in the Early vs. Late Intervention Trial with Estradiol (ELITE). Nine metabolic biomarkers were assessed. Metabolic clusters were compared by early- vs. late-menopause, and correlated with cognitive performance measured at 3 points over a five-year period. Metabolic clusters were also compared longitudinally between women randomized to hormone therapy (HT) vs. placebo, to investigate the effects of HT usage on metabolic biomarkers as well as cognitive function. Metabolic variables measured at baseline generated three distinct clusters. Women in one cluster had a healthy metabolic profile; women in the second cluster were characterized by high blood pressure; and women in the third cluster had an overall unhealthy metabolic profile. Metabolic biomarkers within all profiles were very stable and significantly differed among clusters over the five years of the trial. At baseline, women in the unhealthy metabolic cluster showed a trend towards worse performance on tests of verbal memory than women in the healthy cluster ($p < 0.07$). Women in all clusters showed improvement in cognitive testing over five years, although women with high blood pressure had the least improvement. Longitudinal changes in cognitive function differed significantly between women in early and late menopause on select neuropsychological tests. Analyses of HT effect on cognitive function between clusters are in progress. Outcomes of these analyses will provide rapidly deployable biomarker profiles of developing an at-AD-risk phenotype.

Disclosures: J.R. Rettberg: None. **H. Dang:** None. **H. Hodis:** None. **R.D. Brinton:** None. **W. Mack:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.08/J3

Topic: C.05. Aging

Support: NIA Grant P01AG026572

NIH Grant U01 AG024904

DOD Grant W81XWH-12-2-0012

Title: Screening for Alzheimer's: Identifying at-risk individuals by applying predictive analytics to plasma measures

Authors: *B. C. RIEDEL[1], S. K. MADSEN[1], W. J. MACK[2], P. M. THOMPSON[1], R. D. BRINTON[1];
[1]Neurosci., [2]Preventive Medcine, USC, Los Angeles, CA

Abstract: Alzheimer's disease (AD) affects 5.3 million Americans, with numbers projected to grow as the population ages. As the prodromal phase of AD begins decades before the clinical manifestation of dementia, the detection of early biomarkers is crucial in identifying at-risk populations. Age-associated loss of sex-hormones is concomitantly correlated with an increased incidence of conditions that adversely affect whole-body metabolism, including visceral obesity, insulin resistance, the metabolic syndrome, and diabetes. Longitudinal studies show that cardiovascular risk factors that are associated with these conditions, such as hypertension, hypertriglyceridemia, hypercholesterolemia, and inflammation are associated with an increased risk of AD. While these metabolic markers have all individually been linked to an increased risk for cognitive decline and AD, statistical significance for each has only been reached in population-wide studies. As an initial test to overcome this challenge and determine risk at the individual patient level, K-means cluster analysis was performed on postmenopausal women enrolled in ELITE (Early versus Late Intervention Trail with Estradiol) using measures of plasma insulin, glucose, β-hydroxybutyrate, triglycerides, hemoglobin A1c, LDL and HDL cholesterol, and systolic and diastolic blood pressure. We were able to group cognitively normal healthy individuals into three distinct metabolic clusters, each associated with different risk for cognitive decline (Rettberg, Society for Neuroscience, 2014). To extend this finding to risk of conversion to AD, we applied classification and regression tree (CART) analysis to data from the Alzheimer's Disease Neuroimaging Initiative (ADNI). CART analysis, a tree-building technique, was selected as it can lead to intuitive clinical decision rules by uncovering complex interactions

among predictors not readily discovered with traditional multivariate techniques. Preliminary analyses of ADNI1 participants (N=206) reveal that baseline levels of cholesterol, triglycerides, and pulse pressure are among the earliest branches in the tree, serving to differentiate stable cognitively normal participants (N=160) from those who progress to mild cognitive impairment (MCI) or AD (N=46). Imaging analysis is currently underway, to relate these risk predictors to baseline brain structural differences between the individuals that convert to MCI or AD and age- and sex-matched cognitively normal controls.

Disclosures: B.C. Riedel: None. **S.K. Madsen:** None. **W.J. Mack:** None. **P.M. Thompson:** None. **R.D. Brinton:** None.

Poster

698. Physiology and Pathophysiology of Hormones

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Topic: C.05. Aging

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Sage Therapeutics, Cambridge MA supported the mouse pharmacokinetic LC-MS/MS data acquisition.

Title: Allopregnanolone preclinical therapeutic development in relevant aging and Alzheimer's disease models

Authors: *R. W. IRWIN[1], C. M. SOLINSKY[2], C. M. LOYA[3], K. E. RODGERS[2], G. BAUER[4], M. ROGAWSKI[4], R. D. BRINTON[2];
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Abstract: Allopregnanolone (Allo) is in development as a novel regenerative therapy to treat Alzheimer's disease and other neurological disorders. Allo, allosterically modulates GABA-A receptor chloride channels to stimulate the natural repair machinery of the CNS. With once

weekly pulsatile dosing, Allo was shown to increase neurogenesis, oligodendrogenesis, white matter generation and cholesterol homeostasis while reducing beta-amyloid burden and neuroinflammation. To estimate the safe starting dose range and infusion rate for humans, preclinical and existing clinical Allo studies were compiled. For initial PK studies in humans, fully bioavailable IV infusion was selected. PK analyses of intravenous Allo in rabbit and mouse indicated peak plasma and brain levels (3-fold brain/plasma ratios) at 5min sufficient to activate neuro-regenerative responses at sub-sedative doses. By comparison, slow-release SC suspension of Allo displayed 5-fold brain/plasma ratio at Cmax at 30min. At therapeutic doses by either IV or SC routes, Allo mouse plasma levels ranged between 34-51ng/ml at 30min, comparable to published endogenous human level in the third trimester of pregnancy. Neurogenic efficacy was achieved with Allo brain exposure of 300-500 hr*ng/g. Exposure to Allo, administered within a safe and tolerable dosage range, increased hippocampal markers of neurogenesis in young 3xTgAD and aged wildtype mice. Proliferation marker PCNA was significantly upregulated within 4-24h by Allo IV, TD, IN, IM, and SC routes. IV Allo 1.5mg/kg transiently and robustly phosphorylated CREB within 5min and increased levels of neuronal differentiation transcription factor NeuroD within 4h. IM Allo 2mg/kg in SBECD at 1:6 molar ratio increased PCNA 55% in young 3xTgAD mice at 24h, comparable to SC suspension 10mg/kg. In rats, the maximally tolerated dose of Allo was 8mg/kg for both subcutaneous and intramuscular routes and 2mg/kg for intravenous route accompanied by 30min, 45min, and 10min maximal sedation respectively. Compared to females, males exhibited ≥40% greater sedation behavior time. Allo formulated in SBECD at 1:6 complexation resulted in optimal, by rate and efficacy, Allo delivery to brain by all routes of administration and will advance to clinical trials for Alzheimer’s disease.

Disclosures: **R.W. Irwin:** None. **C.M. Solinsky:** None. **C.M. Loya:** A. Employment/Salary (full or part-time):; Employee of Sage Therapeutics. **K.E. Rodgers:** None. **G. Bauer:** None. **M. Rogawski:** 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 Department of Defense under award number W81XWH-09-1-0746 to MAR. Views and opinions of, and endorsements do not reflect those of the US Army or the Department of Defen. **R.D. Brinton:** Other; Patents pending on allopregnanolone as a therapeutic for mild cognitive impairment and Alzheimer's disease..

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.10/J5

Topic: C.05. Aging

Support: NIA 5P01AG026572

Title: Transcriptional response of estrogen receptors to estrogen across the peri-menopause transition in rat cortical astrocytes

Authors: *E. BACON, E. A. HERNANDEZ, F. YIN, R. D. BRINTON, T. E. MORGAN, C. E. FINCH;
USC, Los Angeles, CA

Abstract: The mission of our Peri-menopause in Brain Aging and Alzheimer's Disease Program Project (P3) is to discover the biological transformations that occur in the aging female brain during the perimenopausal transition that can result in phenotypes predictive of risk for development of alzheimer disease (AD). Using a rat model of the peri-menopause, we have reported previously that cortical astrocytes undergo a change in inflammatory response to estrogen (E2) during transitions from regular to irregular cycling (Arimoto et al 2013). A transcriptional basis for this change includes increase ratio of the nuclear receptors ERα: ERβ. To further explore the hypothesis that the loss of transcriptional response to E2 with age is mediated by changes in ER expression, we measured expression of both receptors in cultured mixed glia (astrocytes: microglia, 3:1) after 24h treatment with E2 (0.1 nM), progesterone (P4)(100 nM), or both (E2+P4). We show that both ERs respond to treatment with E2 and P4 in a context dependent manner that is sensitive to different types of media and growth supplements. We also show differences in basal levels of expression of both ERs in non-treated controls depending on the media type and supplements used. We conclude that local cellular environment is not only an important factor in regulating baseline expression of ERs, but can also influence cellular responses to hormone treatment. Epigenetic changes could provide an explanation for the loss of transcriptional response to E2. Modifications in DNA methylation or nucleosome positioning resulting in altered chromatin states are well known mechanisms by which a cell can modify the potential for gene expression in response to changes in its environment. Further experiments will address underlying mechanisms behind this context-dependent response of ER expression and how this may contribute to age-related changes in inflammatory response.

Disclosures: E. Bacon: None. **E.A. Hernandez:** None. **F. Yin:** None. **R.D. Brinton:** None. **T.E. Morgan:** None. **C.E. Finch:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.11/J6

Topic: C.05. Aging

Support: NIA 5P01AG026572 (to RDB)

Title: Impact of perimenopausal transition on bioenergetic and synaptic function in hippocampus of female rat model of human perimenopause

Authors: *F. YIN[1], J. YAO[1], Z. MAO[1], T. MORGAN[2], E. HERNANDEZ[1], H. SANCHETI[1], H. CHENG[2], C. E. FINCH[2], W. MACK[3], E. CADENAS[1], R. D. BRINTON[1,3];
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Abstract: The mission of our Perimenopause in Brain Aging and Alzheimer's Disease Program Project (P3) is to discover the biological transformations that occur in the aging female brain during the perimenopausal transition that can result in phenotypes predictive of risk for development of Alzheimer's disease (AD) pathology. To address these issues, we investigated hippocampal bioenergetic and synaptic function in a rodent model of human perimenopause using 9-10 month-old rats at different stages of ovarian senescence, as exemplified by regular cycler, irregular cycler and constant estrus (Stages of Reproductive Aging Workshop, 2011). For comparison, we also included the following groups of female rats: regular cycles at age 6 month, regular cycles ovariectomized (OVX) at age 10 month, irregular cycles OVX at age 10 month, and acyclic/constant estrus at age 16 month. Our custom gene array data firstly identified the transition from regular cycler to irregular cycler (9-10 month-old) as the stage where the major age-independent decline in brain bioenergetic gene expression occurs during perimenopause. Functional group analyses revealed decreased expression of genes involved in glucose metabolism, mitochondrial biogenesis/dynamics, molecule transport, and redox homeostasis during this regular-irregular transition, which is confirmed by decreased protein levels of key metabolic enzymes including glucose transporter 3 (GLUT3), pyruvate dehydrogenase (PDH) and ATP synthase F1 complex assembly factor 2 (ATPAF2). Ingenuity Pathway Analysis further indicated that differences in expression patterns during perimenopause are regulated by the upstream insulin/IGF1 and PGC1α signaling pathways. Consistently, biochemical analyses manifested that the regular-irregular transition is associated with compromised mitochondrial function in terms of decreased respiratory capacity, increased production of H2O2, and decreased activity of key bioenergetic enzymes. More importantly, the above-mentioned changes during the transition were accompanied by a decline in functional outcomes, including decreased brain glucose uptake (FDG-microPET) and decreased synaptic plasticity (long-term potentiation). Finally, our data also showed that ovariectomy initiated on regular or irregular cyclers elicited opposed effects on bioenergetic gene expression. Together, these data from a

rodent model of perimenopause provide novel mechanistic insights into the effects of reproductive aging on bioenergetic and synaptic function, which is critical for understanding the perimenopausal transition in both normal brain aging and the development of phenotype of AD risk.

Disclosures: **F. Yin:** None. **J. Yao:** None. **Z. Mao:** None. **T. Morgan:** None. **E. Hernandez:** None. **H. Sancheti:** None. **H. Cheng:** None. **C.E. Finch:** None. **W. Mack:** None. **E. Cadenas:** None. **R.D. Brinton:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.12/J7

Topic: C.05. Aging

Support: Kenneth T and Eileen L Norris Foundation to RDB

Title: Allopregnanolone promotes neural stem cell differentiation to neurons

Authors: *S. CHEN, J. YAO, K. WONG, R. D. BRINTON;
Sch. of Pharm., USC, Los Angeles, CA

Abstract: Our previous studies demonstrated that allopregnanolone (Allo), an endogenous neurogenic steroid, increased proliferation of both rodent and human neural stem/neural progenitor cells (Wang et al., 2005). We also demonstrated that Allo promotes neurogenesis and reversed learning and memory deficits in 3-month-old male triple transgenic mouse model of Alzheimer's disease and in late-stage normal aging mouse (Wang et al., 2010, Singh et al., 2011, Brinton 2013). Recently, we demonstrated that adult neural stem cell (NSCs) exhibited an age- and Alzheimer's- related decrease in proliferative capacity and decrease in neural differentiative capacity (decreased ratio of neuron to astrocyte differentiation) (SfN 2013, 654.13). In this study, we investigated the impact of Allo on neural stem cell differentiation. For *in vitro* analyses, free-floating neurospheres from hippocampus of fetal rat or adult mouse brain were cultured in proliferation medium. The subsequent neurospheres or isolated neural stem cells were plated on coverslips in differentiation medium and treated with Allo (100nM) or vehicle for 7 days. Differentiation of neural stem cell was assessed using neuronal and astrocytic specific markers, MAP2 and GFAP respectively. Allo treatment significantly increased MAP2-positive cells - neuronal differentiation relative to GFAP-positive cells -astrocytic differentiation. In embryonic derived NSCs, the ratio of neurons to astrocytes increased from 1.7:1 in vehicle treated NSCs to 2.2:1 in Allo treated NSCs. In adult derived neural stem cells, the ratio of neurons to astrocytes

was significantly increased from 1:1 to 2:1 with Allo treatment. We further investigated the efficacy of Allo to promote neuronal differentiation of neural stem cells in 5-month-old male triple transgenic Alzheimer's mice. Two-once per week treatments of Allo (10mg/kg) significantly increased expression of early stage neurogenesis marker, doublecortin, by 30%. Flow cytometry-based analysis newly generated cells indicated that Allo-induced a 20% increase in BrdU and NeuN double positive nuclei. Collectively, our data indicate that allopregnanolone promotes neuronal differentiation from neural stem cells. These findings contribute to the translational basis for developing allopregnanolone as a pleiotropic systems level therapeutic to promote the regenerative capacity of the brain (Brinton 2013).

Disclosures: S. Chen: None. **J. Yao:** None. **K. Wong:** None. **R.D. Brinton:** None.

Poster

698. Physiology and Pathophysiology of Hormones

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Program#/Poster#: 698.13/J8

Topic: C.05. Aging

Support: NIA P01AG026572; PERIMENOPAUSE IN BRAIN AGING AND ALZHEIMER'S DISEASE (RDB): Animal Core (TEM); Analytic Core (EC).

Title: Female rat model of human perimenopause documents reductions of key neurosteroids in cortex

Authors: E. A. HERNANDEZ[1], F. YIN[1], D. CARUSO[3], R. C. MELCANGI[3], C. PIKE[2], E. CADENAS[1], R. D. BRINTON[1], C. E. FINCH[2], *T. E. MORGAN[2];
[1]Sch. of Pharm., [2]Davis Sch. of Gerontology, USC, Los Angeles, CA; [3]Dept. of Pharmacol. and Biomolecular Sci., Univ. of Milan, Milan, Italy

Abstract: The mission of our Perimenopause in Brain Aging and Alzheimer's Disease Program Project (P3) is to discover the biological transformations that occur in the aging female brain during the perimenopausal transition that can result in phenotypes predictive of risk for development of Alzheimer's disease (AD) pathology. Core B provides Sprague-Dawley rats of defined ovarian cycling status as a model for the human perimenopausal transition. In humans and lab rodents, as follicle numbers decline below a critical number, cycles become progressively irregular (IRREG) and ultimately cease during middle-age, but with wide individual variations. Although the clinical term menopause does not apply to rodents, which

lack a menstrual flow, some markers of rodent reproductive senescence correspond to stages of menopause defined in STRAW (Stages of Reproductive Aging Workshop, 2011) (Harlow SD 2011 J Clin Endo Metab; Finch CE 2013 J Steroid Biochem). In young rats, regular (REG) 4-5 d estrus cycles prevail from 4- 8 mo. Cohorts of 8 mo retired breeders were followed by longitudinal vaginal cytology (daily lavage) to document their individual cycling profile as REG, IRREG (6-8 d cycles), or acyclic (AC, >9 d), defined by constant estrus (CE) or constant diestrus (CD). At 9 mo, we observed 60% REG, 37% IRREG, 3% AC-CE. By 10 mo the distribution changed to 38% REG, 50% IRREG, 12% AC-CE. By 16 mo, all rats were either AC-CE (47%) or AC-CD (53%). At defined ovarian stages, rat cerebral cortex was analyzed for estradiol, progesterone and its metabolites, and testosterone by mass spectroscopy. In cortex, 17-ß-estradiol gradually decreased from 0.06+0.01 pg/mg, REG-6mo to 0.04+0.01, REG-10mo & IRREG-10mo, to below the detection limit (0.02 pg/mg) at CE-10mo. In addition to estradiol, we observed age-related decreases of several steroids in cortex, including isopregnanolone, dihydroprogesterone, tetrahydroprogesterone, and testosterone. Importantly the decline in these steroids occurred during the perimenopausal transition. For example, at the transition of REG-10m to IRREG-10mo, a >50% reduction occurred in tetrahydroprogesterone (0.83+0.21 to 0.30+0.07 pg/mg, $p<0.02$). These reductions in select steroids during the premenopausal transition predict strong effects on pathways of neurogenesis, APP processing, bioenergetics, and inflammation which are being examined with gene and protein profiling. Together, these data from a rodent model of perimenopause provide novel insights into the effects of reproductive aging on brain functions, which is critical for understanding the perimenopausal transition in both normal brain aging and the development of phenotype of AD risk.

Disclosures: **E.A. Hernandez:** None. **T.E. Morgan:** None. **F. Yin:** None. **D. Caruso:** None. **R.C. Melcangi:** None. **C. Pike:** None. **E. Cadenas:** None. **R.D. Brinton:** None. **C.E. Finch:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.14/J9

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSERC PGS-D3 KGO

NSERC grant 262087 GJT

NSERC grant 46292 AJM

Title: A characterization of proctolin's function as a hormone, a co-transmitter and a putative behavioural role in *Drosophila* melanogaster

Authors: *K. G. ORMEROD, G. J. TATTERSALL, A. J. MERCIER;
Biol. Sci., Brock Univ., St. Catharines, ON, Canada

Abstract: Communication between neurons is the cellular basis for thought and controlled movement, and involves release of neurotransmitters at chemical synapses. Neuropeptides can be released at synapses as co-transmitters, and they can be released into circulation as neurohormones. Here we exploit the technical benefits offered by *Drosophila* third-instar larvae to characterize and distinguish the roles of the neuropeptide, proctolin (RYPLT), as a co-transmitter from its hormonal effects. We demonstrate that bath application of proctolin, which simulates hormonal release, induces sustained contractions in body wall muscles independently of neural activity. This effect is dose-dependent and requires proctolin receptor expression in muscle cells, confirming its postsynaptic nature. We show that proctolin also augments neuronally-evoked contractions in a dose-dependent manner. The magnitude of this proctolin-induced modulation depends on the frequency of neural stimulation, and the threshold for modulation decreases at high stimulus frequencies, which are known to release proctolin from motor neurons. Since proctolin is reported to be present in a subset of motor neurons, our data suggest that its release as a co-transmitter may enable modulation of selected synapses at low hormone concentrations. We have begun to explore behavioural ramifications of altering expression of the proctolin receptor (ProcR) in muscle cells, neurons and ubiquitously in larvae and adult flies. We find a significant reduction in preferred temperature in larvae as a consequence of lowering ProcR expression in muscle fibers. We also demonstrate that lowering ProcR expression has a significant effect on adult geotaxis, indicating an effect on adult motor-function. Thus, proctolin expression also has a profound effect on the behaviour of the larval and adult forms of *Drosophila*.

Disclosures: K.G. Ormerod: None. **A.J. Mercier:** None. **G.J. Tattersall:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

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Program#/Poster#: 698.15/J10

Topic: C.05. Aging

Support: NIH R01-AG034206 (HMBB)

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the Ellison Medical Foundation [AG-SS-2376-09 (HMBB)]

the Glenn Foundation for Medical Research (HMBB)

Title: Effect of insulin-like growth factor-1 on thioredoxin and glutaredoxin systems in growth hormone receptor knockout mice

Authors: *L. ROJANATHAMMANEE[1,2], S. RAKOCZY[2], H. M. BROWN-BORG[2];
[1]Inst. of Sci., Suranaree Univ. of Technol., Nakhon Ratchasima, Thailand; [2]Basic Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: Growth hormone receptor knockout (GHRKO) mice are growth hormone (GH) resistant due to the targeted disruption of the GH receptor/binding protein thus preventing the GH from binding and exerting downstream effects. They live longer than their wild type (WT) controls. Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) have been shown to affect processes involved in cellular stress defense, aging and longevity. Reduced GH/IGF-1 and insulin signaling is associated with enhanced antioxidative capacity and stress resistance, both of which are thought to contribute to lifespan extension. This study was designed to study the possible mechanisms underlying GH's action on cellular stress defense. WT or GHRKO mice were treated with saline or IGF-1 (WT saline, GHRKO saline, GHRKO IGF-1) two times daily for seven days. Thioredoxin (Trx) activity, protein and gene expression were determined. We found that Trx1, Trx2, TrxR1 and TrxR2 mRNA levels were significantly higher in GHRKO as compared to WT mice and IGF-1 treatment suppressed the expression of each. We also found that Grx2 mRNA and cytosolic Grx activity were higher in GHRKO mice. These results suggest that the lack of GH action affects the regulation of thioredoxin and glutaredoxin, factors that regulate translational modification of the proteins and redox balance, thereby further influencing stress resistance.

Disclosures: L. Rojanathammanee: None. **S. Rakoczy:** None. **H.M. Brown-Borg:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.16/J11

Topic: C.05. Aging

Title: Effects of phytoestrogen treatment on the gabaergic system in the retina of ovariectomized mice

Authors: E. VIOLARI, *E. N. YAMASAKI;
Dept. of Life and Hlth. Sci., Univ. of Nicosia, Nicosia, Cyprus

Abstract: Menopause, which is characterized by very low circulating levels of estradiol, is part of the normal ageing process and can constitute up to one third of a woman's life. It could have an additive effect on the decline of cognitive abilities that is seen in normal ageing, and postmenopausal estrogen administration improves performance in memory testing, besides being postulated to potentially slow decline of cognitive functions. Despite that, hormone replacement therapy (HRT) is only indicated for the treatment of the vasomotor symptoms of menopause, and for a short period. The use of readily available over-the-counter dietary supplements containing phytoestrogens and herbal preparations have become popular because of their structural and functional similarity to the natural hormone, and without the adverse effects associated with HRT. Studies have been done which found that phytoestrogens have a small effect on menopause symptoms, decrease the risk of breast and endometrial cancer, and of Alzheimer's disease, and have some positive effects on the cognitive function of younger postmenopausal women. While it is known that in the brain, estradiol has a neuroprotective and neurotrophic effect, and also modulates GABAergic, cholinergic and glutamatergic neurotransmission, the neurobiological mechanisms for the effects seen with phytoestrogen in menopausal women have not been fully studied. The purpose of this study is to characterize the effects of phytoestrogens (genistein and daidzein) on the GABAergic system in the retina of ovariectomized 5 month-old female CD1 mice. Two weeks following ovariectomy, animals were fed daily for 40 days with a drug combination (0.15mg/day genistein and daidzein) or the vehicle mixed with a hazelnut chocolate spread (Nutella®). Following drug treatment, eyes were processed for immunohistochemistry or western blot using antibodies against GAD (ab1511, Chemicon) and GABA (Sigma). Our results show that phytoestrogen treatment increased GAD67 but not GAD65 levels to about 15% above the control. GAD immunoreactivity in the inner plexiform layer increased in the retina of animals treated with the phytoestrogen combination, and GABA immunostaining in the retinas of treated animals was also increased. In this mouse model of menopause, differently from estradiol, phytoestrogen administration modulates positively GABAergic neurotransmission, and it is GAD67 but not GAD65 expression that is being differentially affected.

Disclosures: E. Violari: None. **E.N. Yamasaki:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.17/J12

Topic: C.05. Aging

Support: FAPESP

CNPq

USP (NAP)

Title: Evaluation of serotonin and noradrenalin contents in the amygdala and hippocampus in an animal model of perimenopause

Authors: *N. PESTANA[1], C. M. LEITE[2], R. O. CAROLINO[2], J. A. ANSELMO-FRANCI[2]; [1]Fisiologia, Univ. de São Paulo-Faculdade de Medicina de Ribeirão Preto/FMRP, Ribeirao Preto, Brazil; [2]Morfologia, Fisiologia e Patologia Básica, Faculdade de Odontologia de Ribeirão Preto, Ribeirão Preto, Brazil

Abstract: Perimenopause is a period of transition from reproductive to non-reproductive life in females, usually characterized by neuroendocrine, metabolic and behavioral instability due to a reduction in the number of ovarian follicles. It is a period marked by the appearance of a variety of symptoms that impair the quality of women's life. Clinical studies have identified an increased vulnerability to depression in women during this period compared to other periods of life. Administration of the chemical VCD is an excellent model to experimentally induce perimenopause in female rodents because it accelerates the natural process of follicular atresia with a progressive depletion of primordial and primary follicles. Serotonergic and noradrenergic systems are the major neurotransmitters systems involved in the emotional disturbances, thus, this study aimed to investigate the content of noradrenaline (NA) and serotonin (5HT), and their metabolites, in brain areas (amygdala and hippocampus) involved in the emotional control in this model of perimenopause. Female rats (28 day old) were injected sc daily with VCD (160mg/Kg) or oil for 15 consecutive days. Approximately 80 days after the first injection, the rats were decapitated in the morning of proestrus and diestrus. The brains were removed and the amygdala and hippocampus microdissected. The NA,5-HT, and their respective metabolites (MHPG, and 5-HIAA) contents were measured in the amygdala and hippocampus by High-Performance Liquid Chromatography/ Electrochemical Detection (HPLC/ED). The 5-HT content was significantly reduced in both phases of the estrous cycle in the amygdala and the hippocampus while 5-HIAA was lower only in the amygdala during the diestrus phase. On the other hand, the content of NA and MHPG in both areas was not affected by the follicular depletion induced by

VCD in any of the studied groups. The reduction of 5-HT content observed in this study can be used as a strong basis to reinforce behavioral data and clinical studies regarding the neurophysiologic changes that women experience during this period, especially as related to emotional disorders of depression and anxiety.

Disclosures: **N. Pestana:** None. **C.M. Leite:** None. **R.O. Carolino:** None. **J.A. Anselmo-Franci:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.18/K1

Topic: C.05. Aging

Support: Fulbright Scholarship JSD Program

Title: The implication of melatonin in the improvement of neuronal mitochondria function

Authors: ***A. J. IDOWU**[1], I. I. OLATUNJI-BELLO[1], Y. BAI[2];
[1]Dept. of Physiol., Lagos State Univ. Col. of Med., Ikeja, Lagos, Nigeria; [2]Cell. and Structural Biol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: The changes in mitochondria bioenergetics play a critical role in neuronal function with age. Mitochondria from synaptosomes in the cerebral cortex of young (6 months), middle-age (13 months) and old (26months) mice were isolated and transferred to mitochondria DNA-less LL/2-m21 cell line (rho-zero) to generate cybrids. Mitochondria bioenergetics studies of the cybrids show that there was significant reduction with age in mitochondria membrane potential (MMP), $P<0.05$, in the young, middle-aged and old synaptosomal mitochondria bearing cybrids respectively. The adenosine triphosphate (ATP) levels were also significantly lower in the old mitochondria bearing cybrids compared with middle age and young cybrids ($P<0.05$). Pre-treatment with 500μM melatonin in glucose media replaced with galactose media containing 500μM melatonin after six hours lowered MMP in young cybrids S-Y-24 ($P<0.05$), but increased MMP in the middle-aged mitochondrial bearing cybrid S-M-29, ($P<0.05$). In the old cybrids S-O-48, melatonin pre-treatment at a concentration of 1mM in glucose media replaced with galactose media containing 1mM melatonin after six hours significantly improved cell viability and restored MMP ($P<0.05$). ATP measurements in the young cybrids show that there was no significant difference in ATP levels between the melatonin treated cybrids and untreated cybrids ($P>0.05$). The young mitochondria bearing cybrids were able to produce ATP optimally in the galactose medium showing they have functional mitochondria. Furthermore, ATP level was

significantly increased in the middle-aged mitochondria bearing cybrids, S-M-29, and old mitochondria bearing cybrids, S-O-48, with melatonin treatment. These results establish a strong relationship between melatonin and mitochondria function. It also gives support to other evidences that melatonin may be a potential therapeutic mitochondria target in neuronal dysfunction during normal aging.

Disclosures: A.J. Idowu: None. **I.I. Olatunji-Bello:** None. **Y. Bai:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.19/K2

Topic: C.05. Aging

Support: 5R01MH093723-03

Title: Somatostatin is hypermethylated in the aged human prefrontal cortex

Authors: *B. C. MCKINNEY, C.-W. LIN, H. OH, D. LEWIS, G. TSENG, E. SIBILLE; Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Study: Somatostatin (SST) expression in the human prefrontal cortex exhibits a progressive decrease with aging. Though decreased SST expression in the prefrontal cotex of individuals of advanced age has been consistently demonstrated, the mechanism by which this decrease in expression occurs is unknown. Here, we investigate the contribution of DNA methylation to the regulation of SST expression in aging. Methods: Genomic DNA was prepared from the prefrontal cortices (areas BA11 and BA47) of postmortem brains from twenty younger individuals (age less than 40) and twenty older individuals (age greater than 60), the older group was enriched for individuals exhibiting particularly low levels of SST expression. Genomic DNA was then treated with bisulfite and bisulfite-specific PCR amplification was performed on of the 5' region of SST in a real-time thermocycler. The amplified bisulfite modified DNA was then heated and the temperature at which half the amplicons melted (T50) calculated using fluorescence data from the theromcycler. Results: The T50 of amplicons produced from older individuals is significantly higher compared to the T50 from younger individuals. Conclusion: The 5' region including areas surrounding the transcriptional start site, first exon, and intron of SST is hypermethylated in DNA isolated from the prefrontal cortex of individuals of advanced age suggesting that DNA hypermethylation may contribute to the low levels of SST expression

observed in the brains of older individuals. Significance: Because expression of SST is decreased in the brains of individuals with advanced age, understanding how SST expression is regulated in the brain is critical to understanding the pathology of brain aging and developing interventions to prevent and treat brain aging. This study suggests that DNA methylation may be one mechanism by which SST expression is regulated in the aging human brain.

Disclosures: B.C. McKinney: None. **E. Sibille:** None. **D. Lewis:** None. **H. Oh:** None. **G. Tseng:** None. **C. Lin:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

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Program#/Poster#: 698.20/K3

Topic: C.05. Aging

Support: EY021716

Title: Hippocampal epigenome and aging: Sex and age regulation of DNA methylation

Authors: *W. M. FREEMAN[1], C. MANGOLD[2], D. STANFORD[3], B. WRONOWSKI[3], D. MASSER[3];
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Abstract: Changes in gene expression with aging in the CNS and other tissues are well documented. Recent reports suggest that epigenetic changes, and DNA methylation at cytosine bases in particular, occur with aging. This opens the possibility that epigenetic changes with aging are a central regulatory mechanism of age-related changes in gene expression. However, comprehensive analyses of DNA methylation changes across the genome with aging in the brain and their effects on gene expression have not been performed. A paired analysis of DNA methylation by a novel capture probe based approach and mRNA expression by microarray analysis was performed on Young (3M) and Aged (24M) C57Bl6 male and female mouse hippocampus. The advantage of this new bisulfite sequencing capture approach is that all of the promoter regions in the mouse genome and CpG islands are isolated and sequenced at high depth for quantitative accuracy. These data provide a high resolution analysis of DNA methylation in a neural tissue. Cytosine methylation levels were highest in CG dinucleotide contexts with much lower average methylation levels evident in CHG and CHH contexts. In promoter regions methylation was lowest close to the transcription start site and tended to be higher in the more distal promoter. As well, gene expression levels with any given sample were inversely related to

promoter methylation level. These findings provide a first of their type insight into DNA methylation in the murine CNS. Large numbers of individual cytosine bases were differentially methylated with aging (both hypo- and hyper-methylation). These changes in methylation levels in promoter regions tended to be inversely related to changes in the gene expression of the specific gene. Evident, but more subtle, were differences between sexes within an age and between sexes with aging. Globally these changes in DNA methylation suggest that alterations in methylation levels are directed to specific regions of the genome by a yet to be determined mechanism or mechanisms rather than by a random process or a global shift towards hypomethylation.

Disclosures: W.M. Freeman: None. **C. Mangold:** None. **D. Stanford:** None. **B. Wronowski:** None. **D. Masser:** None.

Poster

698. Physiology and Pathophysiology of Hormones

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 698.21/K4

Topic: C.05. Aging

Support: French National Agency for research (PNRA 007).

Title: A Highfat/highfructose diet induced oxidative damage in the frontal cortex and impaired the redox signaling response under an acute restraint stress

Authors: M. OSMAN[1], C. BATANDIER[1], *N. A. CRIVELLO[2], T. POYOT[3], K. COUTURIER[1], F. CANINI[3], I. HININGER-FAVIER[1];
[1]Natl. Inst. for Health, Joseph Fourier Univ., Grenoble, France; [2]JM USDA Human Nutr. Res. Ctr. On Aging At Tufts Univ., Boston, MA; [3]Army Inst. for Res. in Biol., Grenoble, France

Abstract: There is a large body of evidence linking insulin impairment and cognitive decline. However, the specific effects of unhealthy diet on frontal cortex and on brain mitochondria are poorly studied. We hypothesized that whole-body insulin and glycemia impairment might contribute to oxidative stress in the CNS, which, in turn, might be implicated in cognitive decline. Furthermore, as it is well-known that emotional stress triggers a shift in metabolism leading to glucocorticoid release, we have hypothesised that the oxidative response will be highest in high fat, high fructose (HF/Hfr) rats under an acute emotional stress. Rats (n=30 rats per group) were fed with control (C, healthy) or HF/Hfr diet, and submitted or not to a restraint

stress (RS) (n=10 rats) 30 minutes before they were sacrificed. The effects of both diet and acute emotional stress were evaluated by monitoring redox status [Total antioxidant capacity (FRAP assay), Thioredoxine Reductase (TrxR), glutathione reduced (GSH) and oxidized (GSSG), and oxidative markers on protein (carbonyl), lipid (TBARS) and DNA damage (comet assay)]. The gene expression of proteins belonging to antioxidant defense (Peridoxin2 (PRX2), Thioredoxine2 (TXN2), Glutathione Synthetase (GSSG) were monitored by RT-PCR. After 12 weeks, while the total weight did not differ between the groups, HF/HFr fed rats displayed hyperglycemia, and insulin was positively correlated to TBARS (r=0.42; P=0.02). Furthermore, HF/Hfr group dysplayed oxidative stress on frontal cortex and brain mitochondria assessed by increased DNA damage (2.68±0.66 vs 3.25±0.64 p=0.02), protein carbonyls (60.56 ±2.35 vs 71.18±4 .11 ; p=0.02) and TBARs (1.48±0.08 vs 1.59±0.09 ; p<0.05). The RS decreased the mitochondrial redox status in the control group (FRAP and TrxR were significantly decreased) but without increasing oxidative damage. However, in HF/HFr group, contrary to our hypothesis, the RS decreased the level of oxidative damage induced by the diet on lipid, proteins and DNA, even if the level of peroxidation was still significantly higher compared to its respective control. This surprising effect induced by RS in HF/Hfr fed rats, did not support our hypothesis, but is in agreement with a significantly increased expression of most of mitochondrial genes of redox signaling (TRXN2, PRX2, GSSG) in this group. In conclusion, these data demonstrated that a moderate chronic unhealthy diet without obesity increased oxidative damage in brain similar to those that has been observed in aging. The redox response induced by acute restraint differs under healthy and unhealthy diet.

Disclosures: **M. Osman:** None. **C. Batandier:** None. **N.A. Crivello:** None. **K. Couturier:** None. **I. Hininger-Favier:** None. **T. Poyot:** None. **F. Canini:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.01/K5

Topic: C.06. Developmental Disorders

Support: FRAXA Research Foundation, grant 2013

Telethon grant GGP13145

Title: Potential role of novel 5-HT_7 receptor agonists as pharmacological tools in Fragile X Syndrome

Authors: *L. CIRANNA[1], L. COSTA[2], L. M. SARDONE[1], M. SPATUZZA[3], S. D'ANTONI[3], C. M. BONACCORSO[4], D. PUZZO[1], M. LEOPOLDO[5], E. LACIVITA[5], M. V. CATANIA[3,4]; [1]Dept. of Bio-Medical Sci., Univ. of Catania, Catania, Italy; [2]Dept. of Clin. and Exptl. Med., Univ. of Messina, Messina, Italy; [3]Inst. of Neurolog. Sci. (ISN), Natl. Res. Council (CNR), Catania, Italy; [4]Lab. of Neurobio., IRCCS Oasi Maria Santissima, Troina (EN), Italy; [5]Dept. of Pharm., Univ. of Bari, Bari, Italy

Abstract: 5-HT_7 receptors for serotonin (5-HT) are expressed in brain structures involved in learning and memory and modulate neuronal excitability as well as glutamate-mediated synaptic transmission. We have recently shown that activation of 5-HT_7 receptors reversed metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD) in wild-type and in Fmr1 KO mice (Costa et al., Biol. Psych. 2012, 72:924-933), a mouse model of Fragile X syndrome (FXS) in which mGluR-LTD is abnormally enhanced, suggesting that 5-HT_7 receptor agonists might be envisaged as a novel therapeutic strategy for FXS. In this perspective, we have studied novel molecules with high binding affinity and selectivity for 5-HT_7 receptors and we have tested their effects on hippocampal synaptic plasticity using patch clamp on acute slices from wild-type and Fmr1 KO mice. We show that novel 5-HT_7 ligands structurally related to LP-211, a potent, selective and brain-permeant 5-HT_7 receptor agonist, behave as agonists at 5-HT_7 receptors and are able to reverse mGluR-LTD in the synapse between CA3 and CA1 pyramidal neurons in the hippocampus from wild-type and Fmr1 KO mice. In addition, we have studied the biological effects of RA-7, a compound arising from *in vivo* metabolism of LP-211; the possible implications for systemic administration of LP-211 will be discussed. We are currently investigating the effects of LP-211 on typical abnormal features of Fmr1 KO mice, namely altered dendrite morphology, sensitivity to audiogenic seizures and learning impairment. Preliminary data suggest that *in vivo* administration of a single dose of LP211 (3 mg/Kg) increases the density of dendritic spines of cortical neurons in both WT and Fmr1 KO mice. Our results provide information about the structure-activity relationship of novel 5-HT_7 receptor agonists and suggest that these molecules might become novel pharmacological tools for the therapy of Fragile X syndrome.

Disclosures: L. Ciranna: None. **L. Costa:** None. **L.M. Sardone:** None. **M. Spatuzza:** None. **S. D'Antoni:** None. **C.M. Bonaccorso:** None. **D. Puzzo:** None. **M. Leopoldo:** None. **E. Lacivita:** None. **M.V. Catania:** None.

Poster

699. Fragile X Syndrome

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.02/K6

Topic: C.06. Developmental Disorders

Support: NIH/NICHD Grant ND067225

NIH/NINDS Grant RR029267

NIH/NINDS Grant NS068408

Title: Altered tonic inhibition in the dentate gyrus in a mouse model of fragile X syndrome

Authors: C. R. HOUSER, X. TONG, Y. CETINA, C. S. HUANG, T. S. OTIS, *Z. PENG; Dept. Neurobiol, David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Numerous alterations in the GABA system have been identified in models of Fragile X Syndrome (FXS), and potential alterations in the δ subunit of the GABAA receptor (GABAAR) that mediates tonic inhibition in several regions of the CNS have attracted considerable interest. As a key regulator of neuronal excitability, tonic inhibition could be particularly important in relation to FXS-associated disorders such as hyperactivity, altered anxiety and increased seizure susceptibility. However, alterations in δ subunit expression in mouse models of FXS appear to vary widely among brain regions and at different stages of development. Little is known about the expression or function of the δ subunit in the dentate gyrus, despite this being a forebrain region where the δ subunit is normally expressed at moderately high levels and where its role in mediating tonic inhibition is particularly important. We have thus initiated immunohistochemical studies of δ subunit localization in this region in male fragile X mental retardation 1 (Fmr1) knockout (KO) mice on a C57BL/6 background, utilizing wildtype (WT) littermates as controls. In the KO mice at 3-5 weeks of age, we observed a moderate but distinct decrease in δ subunit expression in the molecular layer of the dentate gyrus where the δ subunit is localized on dendrites of dentate granule cells. Although a decrease in δ subunit expression is generally expected to be associated with reduced tonic inhibition, other GABAAR subunits could compensate for a loss of this subunit, as has been observed in other conditions. To determine if tonic inhibition was decreased in the dentate gyrus of Fmr1 KO mice, we made whole-cell recordings from dentate gyrus granule cells in adult (2 month) Fmr1 KO and WT littermates. We found a significant reduction of GABAAR-mediated tonic currents in Fmr1 KO mice (6.7 ± 1.4 pA in KO mice compared to 31.1 ± 2.4 pA in their WT littermates, $p<0.001$), without a change in the membrane properties of these cells. Furthermore, we tested sensitivity to the δ subunit-selective agonist THIP (1 μM) and modulation of δ subunit currents by the δ subunit-selective allosteric modulator DS2. Both modulations were decreased in Fmr1 KO granule cells by about 50%. Interestingly, the extent of the functional deficits appeared to be greater than those suggested by the imunohistochemical studies of δ subunit expression in this region. Together the data suggest that GABAAR-mediated tonic inhibition is substantially impaired in dentate granule cells in this model of FXS and that additional factors, such as altered

surface expression, subunit partnerships or intracellular trafficking, could be limiting the function of remaining subunits.

Disclosures: **C.R. Houser:** None. **X. Tong:** None. **Y. Cetina:** None. **C.S. Huang:** None. **T.S. Otis:** None. **Z. Peng:** None.

Poster

699. Fragile X Syndrome

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Program#/Poster#: 699.03/K7

Topic: C.06. Developmental Disorders

Support: Lundbeckfonden

Title: In mature fmr1 ko mice, dhpg-induced ltd is disrupted by synaptically-activated nmda receptors

Authors: ***A. K. TOFT**, N. N. UMAR, T. G. BANKE;
Aarhus Univ., Aarhus C, Denmark

Abstract: Fragile X syndrome (FXS) is caused by a trinucleotide repeat expansion in the X-linked FMR1 gene, which prevents the expression of its encoded protein, Fragile X Mental Retardation Protein (FMRP). We are studying the mouse model of FXS, the Fmr1 KO mouse (Fmr1/y), which demonstrates a significant increase in global protein synthesis in the brain. Two major findings, increased long-term depression (LTD) mediated by mGluR5 receptors and an impaired AMPA receptor LTP, demonstrate a change in synaptic plasticity in the FmR1 KO mouse. However, there is surprisingly little information regarding involvement of NMDA receptors in these processes. We thus investigated whether the function of NMDA receptors was changed in the KO mouse. Using the field potential recording technique, we are studying changes in synaptic plasticity within the CA1 area of the hippocampus. In acute slices from young mice (P30-40), application of the group 1 mGluR agonist DHPG, in the presence of the NMDA receptor antagonist APV, induced slightly more LTD in KO mice than in control mice (68+/-8% vs. 77+/-2%). In the absence of APV, DHPG induced LTD in KO and WT (60+/-8 and 63+/-3, respectively), suggesting that NMDA receptors play an insignificant role in DHPG-induced LTD in both KO and WT mice at this young developmental stage. As with young mice, in slices from mature WT mice (>P60), DHPG-induced LTD was 50+/-4% of baseline in the absence of APV and 62+/-4% of baseline in the presence of APV. In KO mice, in the presence of

APV, DHPG induced LTD (59+/-5% of baseline). This was in sharp contrast to recordings from KO mice, where we found that in the absence of APV, no or only very little LTD induced by DHPG (94+/-7% of baseline). These data strongly suggest that NMDA receptor signaling is disrupted or altered in mature KO mice. We next took advantage of the well-known use-dependent block of NMDA receptors by MK-801. Preapplication of the slice with MK-801 for 10 minutes restored DHPG-induced LTD in slices from mature KO mice. In mature WT slices, MK-801 treatment had no effect on DHPG-induced LTD. Thus, this suggests that synaptically active NMDA receptors in KO mice are disrupting DHPG-induced LTD. We are currently testing different NMDA receptor specific antagonists and downstream kinases/phosphatases on DHPG-induced LTD. Furthermore, we are investigating changes in spine morphology in the CA1 area of the hippocampus. These results suggest a new involvement of NMDA receptors in the pathophysiology of FXS and highlight a novel potential path for treatment for the disease. Thus, based on these findings, antagonizing NMDA receptors could lead to reversal of the FXS phenotype within the CA1 area of hippocampus in mature mice.

Disclosures: **A.K. Toft:** None. **N.N. Umar:** None. **T.G. Banke:** None.

Poster

699. Fragile X Syndrome

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Program#/Poster#: 699.04/K8

Topic: C.06. Developmental Disorders

Support: FRAXA Research Foundation

NIH NS064967

Title: Regulation of protein synthesis by Bcl-xL and FMRP

Authors: ***P. LICZNERSKI**[1], R. J. LEVY[2], E. A. JONAS[1];
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Abstract: We have found previously that Bcl-xL, a member of the Bcl-2 family of proteins, acutely and chronically enhances synaptic connectivity in multiple ways. Bcl-xL- is necessary for certain forms of synaptic plasticity. Bcl-xL alters mitochondrial positioning and metabolism in the synapse and directly affects vesicle trafficking. Our studies suggest that Bcl-xL could play

a role in aberrant development of synapses observed in Fragile X syndrome (FXS). Indeed, studies in two different Fmr1 mutant strains showed increased expression of BcL-xL. The present study focuses on the therapeutic potential of pharmacological inhibitors of Bcl-xL in rodent FXS models. The goal will be to eliminate overabundant and/or aberrant synaptic connections in Fmr1 KO neurons and to test for behavioral improvements that may result from the therapy with Bcl-xL inhibitors. So far we have found that: 1) Inhibition of Bcl-xL by the small molecule inhibitor ABT-737 produces a marked enhancement of protein translation in neurons assayed by Dendra fluorescence (photo-convertible Dendra that has the 3' and 5' UTR of actin). 3) The increased rate of protein translation produced by ABT-737 is comparable to or greater than that produced by FMRP knock out. 4) The increase in protein translation produced by ABT-737 is occluded in the Fmr1-/- mouse neurons, suggesting that inhibition of Bcl-xL or genetic deletion of Fmr1 both produce an effect on protein translation perhaps through the same mechanism. 5) A hallmark of the increase in translation in Fmr1-/- hippocampus is enhanced protein translation-independent LTD. We find that ABT-737 enhances LTD produced by low frequency stimulation or by DHPG in WT rat and mouse hippocampal slices, an effect that is occluded in the Fmr1-/- slices. 6) Published data suggest that Bcl-xL levels are increased in the Fmr1-/- mouse brain. Our new preliminary data suggest that depletion of Bcl-xL by shRNA increases FMRP levels. Our data suggest a novel Bcl-xL dependent signaling pathway regulating protein translation in neurons, that works in parallel to, or in cooperation with, that of FMRP. This would suggest that manipulation of Bcl-xL function may ameliorate Fragile X syndrome.

Disclosures: P. Licznerski: None. **R.J. Levy:** None. **E.A. Jonas:** None.

Poster

699. Fragile X Syndrome

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Topic: C.06. Developmental Disorders

Support: Academy of Finland Grant

Arvo and Lea Ylppö Foundation Grant

Finnish Brain Research Foundation Grant

Title: Defects of glial cell differentiation in the absence of fragile X mental retardation protein

Authors: *M. L. CASTRÉN, H. MONONEN, V. ACHUTA;
Inst. of Biomedicine, Univ. of Helsinki, Helsinki, Finland

Abstract: Fragile X syndrome (FXS) is a common cause of inherited intellectual disability and a well characterized form of autism spectrum disorder. FXS is caused by transcriptional silencing of the *FMR1* gene that encodes the fragile X mental retardation protein (FMRP). FMRP is required for the normal maturation and function of synapses. The absence of FMRP results in developmental changes of glutamatergic and GABAergic systems. An imbalance of neocortical excitation and inhibition is thought to lead to network hyperexitability in FXS. Alterations of FMRP-deficient neural progenitor differentiation *in vitro* have been shown to correlate with abnormalities during brain development and the behavioral phenotype of *Fmr1* knockout (KO) mice, a mouse model for FXS. We previously showed that brain-derived neurotrophic factor (BDNF)/TrkB signaling is involved in the aberrant differentiation of FMRP-deficient progenitors. Cleavage of mature BDNF from its preform is activated by tissue plasminogen activator (tPA)-driven conversion of plasminogen to plasmin whereas plasmin-independent entzymatic activity of tPA potentiates glutamatergic signaling. We have studied the role of glia in the developmental abnormalities found in brain of *Fmr1* KO mice by investigating neuronal migration and differentiation in neurosphere cultures derived from embryonic mouse brain and human patient-specific induced pluripotent stem (iPS) cells. We showed that the expression tPA is increased in glial cells differentiated from FMRP-deficient progenitors and in brain of *Fmr*1 KO mice. In time-lapse imaging, blocking the tPA function by a neutralizing antibody promoted early glial differentiation and reduced the velocity of nuclear movement of FMRP-deficient radial glia. Using intracellular calcium recordings we observed responses to glutamate. Responses to metabotropic glutamate receptor activation by (S)-3,5 dihydroxyphenylglycine (DHPG) were found in radial glial cells. The proportion of DHPG-responsive cells close to the neurosphere cell cluster was increased in differentiated FMRP-deficient neurospheres when compared with wild type controls suggesting defect in the migration and maturation of glial cells in FXS. Furthermore, we found that the morphology of GFAP-immunoreactive astrocytes in differentiating FMRP-deficient neurospheres differed from wild type controls. The results indicate that glial cells contribute to the altered neuronal differentiation in FXS and tPA-mediated mechanisms are involved in functional changes of FMRP-deficient glia.

Disclosures: **M.L. Castrén:** None. **H. Mononen:** None. **V. Achuta:** None.

Poster

699. Fragile X Syndrome

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Topic: C.06. Developmental Disorders

Support: NIH R01 EY022730

UL1TR0000075

GW Undergraduate research fellowship

Title: Disrupted resting state and sensory evoked activity during a critical developmental period in FMR1 mutant rats

Authors: *J. BERZHANSKAYA[1], A. S. GORIN[2], M. A. PHILLIPS[1], M. T. COLONNESE[1]; [1]Pharmacol. & Physiol., [2]SEAS, George Washington Univ., Washington, DC

Abstract: Identification of presymptomatic circuit perturbations in developmental neurological disorders can provide novel intervention targets early enough to prevent the development of disease. We used a model of Fragile X Syndrome in the rat (the Fragile X mental retardation protein 1 mutant (Fmr1KO)), a species for which visual cortical activity development has been carefully matched to that of human neonates, to identify the earliest developmental age at which cortical activity patterns are affected by FMR1 deletion. Extra-cellular recordings using linear multi-electrode arrays were used to extract local field potential and spiking activity in head-fixed, unanesthetized animals during wakefulness. Animals recorded at age equivalents to the human fetal period (P4-11, before eye opening) showed no differences between KO and control. In KO animals eye opening occurred on time and the resting state cortical activity changed from discontinuous rhythmic bursting to continuous desynchronized activity similarly to control. However, from this time period onward (between p16 and p36), FMR1KO rats showed evidence of cortical hyperexcitability, characterized by increased field potential power in the in beta-gamma range and less power in the delta-theta range. This was true both of spontaneous activity during rest as well as for visual evoked activity. Examination of spectral power of the local field during quiet wakefulness, whisking/chewing, or active body movements, shows that network activity in both control and KO animals is dominated by beta-gamma oscillations during and immediately following active movement. The difference between groups arises because KO animals fail to increase slower (1-10 Hz) and reduce beta-gamma oscillations during quiet wakefulness or whisking/chewing. Together our results suggest that heightened network excitability following FMR1 elimination may contribute to the disease by diminishing the capacity of the cortex to deactivate appropriately. This may lead to the sensory hypersensitivity observed in FRX. Our results also indicate that changes in cortical activity caused by FMR1 deletion can be detected electrophysiologically in rodents at age equivalents much earlier than the age of typical diagnosis in humans.

Disclosures: **J. Berzhanskaya:** None. **A.S. Gorin:** None. **M.A. Phillips:** None. **M.T. Colonnese:** None.

Poster

699. Fragile X Syndrome

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Program#/Poster#: 699.07/L2

Topic: C.06. Developmental Disorders

Support: NIH Grant MH097093-01

Title: Abnormal development of network dynamics of *in vitro* Fragile X Circuits

Authors: *H. MOTANIS, D. BUONOMANO;
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Abstract: A challenge in interpreting the diverse set of neural phenotypes observed in animal models of FXS is the potential confound between cause and effect. While it is likely that some phenotypes are causally involved in the neurological abnormalities of FXS it is possible that some of them are indirect consequences of abnormal development and experience. To help disentangle this potential confound we used an *in vitro* developmental approach, and examined the hypothesis that FXS reflects abnormal network regimes produced by deficits in the ability to coordinate the many cellular and synaptic properties that govern network dynamics. First, we performed whole-cell recordings from pyramidal neurons of cortical organotypic slices cultured for 8-30 days. We observed a significant developmental decrease in intrinsic excitability in both WT and KO littermate slices ($p<10-4$). Moreover, neurons of KO circuits exhibited increased excitability compared to WT circuits ($p<0.01$). Network activity was quantified using a number of measures of spontaneous activity: STD of the voltage traces, the number of times the voltage crossed a 5 mV “threshold” above rest, and the frequency of Up states. We found a significant developmental increase in all measures, and a significant genotype and development interaction for STD and Up states frequency ($p<0.01$). Specifically, KO circuits exhibited a significant delay in spontaneous activity development. We also observed a novel phenotype in which Up states within cells of mature KO circuits were more variable ($p<0.01$). We next explored the ability of KO circuits to regulate spontaneous activity in response to “sensory experience” using a homeostatic plasticity protocol. We virally transfected pyramidal neurons with ChR2-EYFP. Chronic optical stimulation for 2 days resulted in homeostatic plasticity in both genotypes: significant reduction in all measures of spontaneous activity ($p<10-3$). There was, however, no

effect of genotype. Together these results indicate that there are robust differences in the *in vitro* development of network activity and dynamics between KO and littermate WT circuits. These observations recapitulate some of the *in vivo* delayed developmental observations. A novel observation was that Up states in mature FXS circuits were less reproducible_possibly meaning that the patterns of activity in FXS circuits were less able to "consolidate" into reproducible trajectories. Our results also indicate that FXS circuits are able to undergo homeostatic plasticity, and we are currently investigating whether homeostatic plasticity has a normal time course and duration.

Disclosures: H. Motanis: None. **D. Buonomano:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.08/L3

Topic: C.06. Developmental Disorders

Support: The FRAXA Research Foundation

Autism Speaks

The National Fragile X Foundation

NIH

Title: Insulin misregulation underlies circadian and cognitive deficits in a *Drosophila* fragile x model

Authors: *S. M. MCBRIDE[1], R. MONYAK[2], D. EMERSON[2], B. SCHOENFELD[2], X. ZHENG[2], D. CHAMBERS[4], P. HINCHEY[5], C. CHOI[6], T. MCDONALD[5], F. BOLDUC[4], A. SEHGAL[3], T. JONGENS[2];
[2]Genet., [3]HHMI, [1]Univ. of Pennsylvania, Philadelphia, PA; [4]Univ. of Alberta, Edmonton, AB, Canada; [5]Mol. Pharmacol., Albert Einstein Col. of Med., New York City, NY; [6]Dermatol., Drexel Col. of Med., Philadelphia, PA

Abstract: Fragile X Syndrome is caused by loss of *FMR1* gene activity and is the most commonly inherited form of cognitive impairment and autism. Patients with this disorder also suffer from hyperactivity, attention deficit disorder, irritability, sleep problems and have noted

neuro-anatomical defects. A *Drosophila* fragile X model, based on loss of *dfmr1* function, displays several relevant phenotypes, including defects in circadian regulation, social interaction (with peers and in naïve courtship), memory and morphology of some neurons. Here we show that the circadian and memory deficits displayed by *dfmr1* mutants are due to enhanced insulin signaling. In a study aimed at mapping the spatial requirements of *dfmr1* activity for normal circadian regulation, we found that select expression of *dfmr1* in the insulin producing cells (IPCs) of the brain is sufficient to restore normal circadian behavior. Surprisingly, this select expression also rescues the memory deficits in the fragile X model. Examination of the insulin pathway reveals elevated levels of *Drosophila* insulin-like peptide 2 (Dilp2) in the IPCs and elevated insulin signaling throughout the *dfmr1* mutant brain. Consistent with elevated insulin signaling being causal in *dfmr1* mutant phenotypes, we found that select expression of *dfmr1* in the IPCs reduces insulin signaling and that genetic reduction of the insulin pathway also leads to amelioration of these phenotypes. Finally we show that treatment with the FDA approved drug metformin can also rescue the memory in two different paradigms. Our results indicate that insulin misregulation underlies the circadian and cognitive phenotypes displayed by the *Drosophila* fragile X model, and thus reveals another pathway that can be targeted by new and current drugs to treat fragile X patients.

Disclosures: **S.M. McBride:** None. **R. Monyak:** None. **D. Emerson:** None. **B. Schoenfeld:** None. **X. Zheng:** None. **D. Chambers:** None. **P. Hinchey:** None. **C. Choi:** None. **T. McDonald:** None. **F. Bolduc:** None. **A. Sehgal:** None. **T. Jongens:** None.

Poster

699. Fragile X Syndrome

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Program#/Poster#: 699.09/L4

Topic: C.06. Developmental Disorders

Support: NIH/NIMH R01 MH092877-01

Title: Aberrant cofilin signaling in a mouse model of fragile x syndrome

Authors: *A. PYRONNEAU, R. ZUKIN;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and autism. The primary cause is an unstable CGG trinucleotide repeat expansion

leading to hypermethylation and transcriptional silencing of the FMR1 gene. The Fragile X Mental Retardation Protein (FMRP), the FMR1 gene product, is an mRNA binding protein, which is known to repress over 1000 mRNA targets. Loss of FMRP causes unchecked translation of many proteins in the brain normally regulated by FMRP. The FXS neuroanatomical hallmark is an overabundance of dendritic spines with a long, thin and immature morphology. Spine defects have been reported in a large variety of neurons throughout the brain including layer V pyramidal neurons of the somatosensory cortex of Fragile X mice. However the molecular mechanisms linking loss of FMRP to aberrant spine morphology remains unclear. A major class of actin cytoskeleton regulators is the Rho family of small GTPases including Rac1, which exhibits elevated activity in the cortex of Fragile X mice. Growing evidence now implicates the actin depolymerizing factor cofilin in the spine defects associated with FXS via the small GTPase, Rac1. Rac1 promotes phosphorylation and inactivation of the actin depolymerizing factor cofilin, leading to enhanced actin polymerization and an increase in spine density and length. The underlying hypothesis of my thesis research is that loss of FMRP causes enhanced Rac1 activity, which in turn phosphorylates and inactivates cofilin leading to enhanced actin polymerization and an overabundance of immature spines. Using western blot analysis we show that Rac1 activity is elevated, Pak1 (a Rac1 effector) exhibits elevated activity, phosphorylation of Slingshot and Lim kinase (two key downstream targets of Pak1 and upstream regulators of cofilin) are elevated, abundance of profilin 2 (an actin polymerizing factor downstream of Rac1) is elevated and cofilin is inactivated in synaptoneurosomes from the somatosensory cortex of Fragile X mice at one (but not four) weeks of age. Moreover we show by the Golgi staining method that dendritic spine density and morphology are abnormal in the somatosensory cortex of Fragile X mice at one week of age. These findings implicate Rac1/cofilin signaling in the spine defects associated with FXS. Ongoing research is focusing on a causal link between the loss of FMRP and aberrant Rac1/cofilin signaling, enhanced phosphorylation/inactivation of cofilin and aberrant spine morphology and density in Fragile X mice.

Disclosures: **A. Pyronneau:** None. **R. Zukin:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.10/L5

Topic: C.06. Developmental Disorders

Support: US Army MRMC #10917352

Title: Impaired olfactory discrimination learning in Fragile X knockout mice

Authors: S. A. KEIL[1], *J. R. LARSON[2];
[1]Grad. Program in Neurosci., [2]Dept Psychiat, Univ. Illinois Chicago, CHICAGO, IL

Abstract: Fragile X Syndrome (FXS), caused by mutation of the FMR1 gene, is the leading cause of inherited intellectual disability and is known to cause cognitive impairment, hyperactivity, attention deficits and seizure disorders. Previous studies using a mouse model for FXS (Fmr1-KO) have described impairments in acquisition of simultaneous-cue olfactory discriminations and a specific deficit in LTP in primary olfactory cortex. The present study tested for impaired olfactory discrimination learning in fragile X mice, using a hippocampus-independent successive-cue training procedure. Male Fmr1-KO and WT littermate mice (C57BI/6J background; 5-12 months old) were trained to criterion (8 consecutive correct responses) in a fully-automated, successive-cue, go/no-go, two-odor discrimination task. Each mouse was trained in 100-trial sessions on eight separate discrimination problems. Each trial consisted of the presentation of one of two discriminative odors. The eight discrimination problems were presented to half of the mice in each group (randomly chosen) in one order, and in reverse order for the other half. Mice were run blind with respect to genotype. Fmr1-KO mice made significantly more errors than WT littermates before acquiring the first discrimination problem. Both WT and Fmr1-KO mice made fewer errors on the remaining discrimination problems and did not differ in this regard. Combined with previous work, the present study indicates that Fmr1-KO mice show similar impairments in learning olfactory discriminations, regardless of whether the discriminative cues are present together (simultaneous-cue task) or separately (successive-cue task) on individual trials. Both paradigms depend on the integrity of piriform cortex but are differentially affected by hippocampal damage. This suggests that the impaired learning in Fmr1-KO mice may be a result of LTP impairments in the primary olfactory (piriform) cortex.

Disclosures: S.A. Keil: None. **J.R. Larson:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.11/L6

Topic: C.06. Developmental Disorders

Support: Intramural Research Program, National Institute of Mental Health

Autism Speaks Postdoctoral Fellowship 8679

Title: Sleep duration in Fmr1 knockout mice

Authors: M. LEVINE, R. REITH, *C. B. SMITH;
Section on Neuroadapatation and Protein Metabolism, NIH, NIMH-SNPM, BETHESDA, MD

Abstract: Sleep disorders are one of the most common concurrent disorders among autism patients, affecting between 50%-80% of these individuals. Interestingly, studies have shown a correlation between both sleep abnormalities and behavioral problems in autistic patients, but the role sleep abnormalities play in contributing to these autistic phenotypes is still unclear. In order to study autism, we focused on the most common known genetic cause of autism, fragile X syndrome (FXS). We studied a mouse model of FXS, the *Fmr1* KO mouse, and we assessed sleep in male *Fmr1* KO and wild type (WT) mice at three weeks of age (adolescents) and two months of age (adults). Sleep duration was measured over a three day period by means of home-cage activity monitors in a 12:12 hr, light:dark environment. Activity was measured in 10s epochs, and 4 consecutive epochs (40s) was scored as sleep. We found a statistically significant reduction in light-phase sleep duration, for adolescents, and total sleep duration, for adults, in *Fmr1* KO mice compared to WT. We attempted to lengthen sleep duration in *Fmr1* KO mice by chronic administration of melatonin, an endogenous circadian regulating hormone. Melatonin was administered in the drinking water from three weeks of age to two months of age, at a dose of 4 mg/kg. Melatonin treatment did not significantly affect sleep duration in either *Fmr1* KO or WT mice; however, melatonin appeared to have had positive effects on circadian rhythm. Currently, we are investigating alternate sleep-promoting compounds and their effects on sleep duration in *Fmr1* KO mice.

Disclosures: **M. Levine:** None. **R. Reith:** None. **C.B. Smith:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.12/L7

Topic: C.06. Developmental Disorders

Title: VEGF inhibition alleviates abnormalities in a mouse model of the fragile X mental retardation syndrome

Authors: *A. BELAGODU, R. GALVEZ;
Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: The Fragile X (FX) Syndrome is the leading form of inherited mental retardation. Although the cause of the syndrome is well understood, transcriptional silencing of fmr1, the mechanism(s) by which this causes neuronal and cognitive abnormalities remains elusive. Our recent anatomical analyses have demonstrated that FX mice exhibit elevated vasculature in primary visual cortex compared to control mice (Galvan & Galvez 2012). One of the most promonent regulators of vascular growth, shown to positively correlate with vascular density is VEGF (Vascular endothelial growth factor) (Neufeld et al., 1999; Ferrara et al., 2003). Our further analyses have demonstrated that VEGF is overexpressed in the neocortex of FX mice, suggesting that excessive neocortical VEGF expression is causing FX vascular abnormalities. However, in addition to regulating vasculature, recent analyses have demonstrated that VEGF can also alter neuronal properties such as increasing axonal growth, facilitating cell survival, and stimulating neurite proliferation (Sondell et al., 1999; Xiao et al., 2007). These VEGF induced neuronal properties are consistent with abnormalities observed in FX (increased axonal material, increased sertoli cell number, and increased number of immature spines). To explore VEGF's potential for mediating FX abnormalities the following study demonstrates that blocking VEGF can alleviate some FX cognitive and behavioral abnormalities. These analyses provide important insight towards the underlying mechanism driving cognitive and neuronal abnormalities in FX.

Disclosures: A. Belagodu: None. **R. Galvez:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.13/L8

Topic: C.06. Developmental Disorders

Title: Corpus callosum abnormalities in a mouse model of fragile X syndrome

Authors: *T. KOBIL-MOAV[1,2], C. SMITH-HICKS[3,2], J. ZHANG[1], G. PELLED[1,2];
[1]Radiology, Johns Hopkins Sch. of Med., BALTIMORE, MD; [2]Kennedy Krieger Inst., Baltimore, MD; [3]Neurol., Johns Hopkins University, school of medicine, Baltimore, MD

Abstract: Autism is a neuro-developmental disorder with multiple etiologic mechanisms and affects 1/68 children in US. Reduced volume of the corpus callosum, the main interhemispheric connective pathway, is a reproducible MRI finding observed in brains of individuals with autism. Callosal projections are known to play key roles in cognition, associative behavior, as well as in motor and sensory regulation. Fragile X syndrome is the most common single gene cause of autism. Fmr1 knockout mice, a model of fragile X syndrome recapitulates many autistic phenotypes, such as impaired socialization, motor dysregulation, increased risk for seizures and cognitive deficits. We hypothesized that Fmr1 knockout mice (KO) may also exhibit deficits in corpus callosum microstructure. To assess this we used non-invasive, ultra-high field preclinical 11.7 Tesla scanner to image white matter characteristics *in vivo*, in 8 weeks old Fmr1 KO (n=5) and aged-matched control C57Bl6 (n=5) mice. We acquired diffusion tensor imaging (DTI) and magnetization transfer (MT) data, which are sensitive to changes in the white matter microstructure and myelin content, respectively, often associated with pathology. For DTI, thirty diffusion (b-value 1500 s/mm2) and 2 non diffusion-weighted images with a field of view= 15 x 15 mm, 0.5 mm thick slices and a matrix size of 128 x 128 (zero-padded to 256 x 256), corresponding to a spatial resolution of 120 x 120 μm were acquired with δ = 3 ms, Δ = 15 ms. Diffusion tensors were calculated using a Log-linear fitting method using DTIStudio and fractional anisotropy (FA) and axial and radial diffusivity maps were calculated from the tensor data. MT images were acquired with a similar field of view and resolution with an offset frequency of -3KHz. MT ratio (MTR) maps were calculated from MT and reference image. Regions of interest were obtained by segmenting the corpus callosum area in the coronal slices of FA and MT images using ROIEditor. The imaging results demonstrated significant ($p<0.05$, two tailed student t-test) changes in FA, radial diffusivity (RD) and MT in the corpus callosum area positioned at the slices at 1-1.5 mm anterior to the bregma: control FA 0.49 ± 0.027, KO FA 0.39 ± 0.031; control RD 4.8 ± 0.37x10^{-4} mm^2/s, KO RD 6 ± 0.38 x 10^{-4} mm^2/s; control MTR 0.59 ± 0.017, KO MTR 0.55±0.024, respectively. Our results demonstrate a loss in the interhemispheric white matter tract structural integrity and myelin content in the Fmr1 knock-out mice in frontal brain areas. These areas are associated with social cognition and motor control, thus implicating the corpus callosum integrity in social cognition and motor dysregulation of fragile X syndrome.

Disclosures: **T. Kobil-Moav:** None. **C. Smith-Hicks:** None. **J. Zhang:** None. **G. Pelled:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

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Program#/Poster#: 699.14/L9

Topic: C.06. Developmental Disorders

Support: Canadian Institute of Health Research

Fragile X Research Foundation of Canada

Title: NMDA receptor hypofunction and dendritic atrophy in the dentate granular cells of Fragile X mice

Authors: S. YAU, J. CHIU, C. CHIU, M. GHILAN, C. BOSTROM, *B. R. CHRISTIE;
Div. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and the major cause of autism spectrum disorders in human. It is caused by over expansion of cytosine-guaine-guanine (CGG) trinucleotide in Fmr1 gene, resulting in complete loss of the fragile X mental retardation protein (FMRP). The dentate graunlar cell layer (GCL), which is with active postnatal neurogenesis, consists of heterogenous neuronal population with gradient ages from the superficial layer to deep layer of the GCL. Activation of N-methly-D-aspartate receptor (NMDAr) is known to play an important role in development and structural plasticity of dendritic arborization and spines during neuronal development. Using Fmr1 knock out mice (Fmr1-/-), we have previously shown a hypofunction of NMDAr specific in the hippocampal dentate region. Here we examined evoked responses to medial perforant path stimulation of whole cell patch clamping and Sholl analysis of dendritic morphology of biocytine-filled neurons from hippocampal slice preparation. Here we show that mature neurons in Fmr1-/- mice did display significantly smaller NMDA currents and a higherα-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to NMDA ratio than wildtype mice. These deficits were associated with significant decrease in dendritic complexity, with both dendritic length and number of intersections being significantly reduced. In contrast, although young neurons from Fmr1-/- mice had a lower level of NMDA current and higher level of AMPA/NMDA ratio they did not show a significant change in dendritic complexity. The data indicate that a loss of FMRP causes NMDA receptor deficits in both young and old neurons, but that deficits in dendritic arborization may only apparent later in life.

Disclosures: S. Yau: None. **J. Chiu:** None. **C. Chiu:** None. **M. Ghilan:** None. **C. Bostrom:** None. **B.R. Christie:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.15/L10

Topic: C.06. Developmental Disorders

Support: Patrick Wild Centre

Autistica

MRC

DBT, India

Title: Fmr1 knockout rats exhibit abnormal development of episodic memory but normal spatial memory in adulthood

Authors: A. ASIMINAS[1,2], S. M. TILL[3,2], A. W. A. BURT[1], E. R. COX[1], R. G. M. MORRIS[1,4], S. CHATTARJI[4], *D. J. WYLLIE[3,2,4], P. C. KIND[3,2,4], E. R. WOOD[1,2];
[1]Ctr. for Cognitive and Neural Systems, [2]Patrick Wild Ctr., [3]Ctr. for Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom; [4]Ctr. for Brain Develop. and Repair, Natl. Ctr. for Biol. Sci., Bangalore, India

Abstract: Fragile X syndrome (FXS) is the leading identified cause of intellectual disability and Autism Spectrum Disorder (ASD). It is caused by epigenetic silencing of the fragile X mental retardation gene (Fmr1), causing a loss of Fragile-X mental Retardation Protein (FMRP). Over the last 2 decades, much has been learned about the pathophysiology related to the loss of FMRP from mouse models of FXS. The recent generation of a rat model of FXS opens the door, not only to validate phenotypes across mammalian species, but also to address cognitive dysfunction using paradigms that are more difficult to address in mice. This study uses the Fmr1 KO rat, to examine the development of episodic-like memory from postnatal day 25 (P25) to adulthood (P71). Sprague Dawley rats (n=16 Fmr1 KO, n=16 WT) were tested in four spontaneous exploration tasks over two days: novel object preference (NOP), object-in-context (OC), object-in-place (OP) and object-place-context (OPC). This two-day procedure was repeated 8 times between P25 and P71 to assess the development of the ability to discriminate novel from familiar objects, and novel from familiar object-context, object-place and object-place-context configurations. WT rats showed a developmental profile of performance similar to that reported in Lister hooded rats, with significant preference for novelty appearing first for NOP, then OC, and finally OP and OPC (Lyon and Langston, British Neurosci. Assoc. Abstr. 22: P1-D-147 2013). Fmr1 KO rats performed similarly to WT rats in the NOP and OC tasks across the developmental time course. In contrast, their ability to discriminate novel from familiar object-place (spatial) and object-place-context (episodic-like) associations was significantly impaired (OP was delayed, and OPC ability did not develop). Spatial memory of adult Fmr1 KO rats was assessed using two different protocols in a water maze: a reference memory and reversal task and

a delayed matching to place task, and also in a delayed forced choice alternation task on a T-maze. Fmr1 KO rats did not differ significantly from controls in any task, suggesting that spatial reference and working memory are intact. The lack of spatial memory deficits in Fmr1 KO rats suggests potentially distinct roles for FMRP in cognition in different mammalian species. These findings, together with the novel demonstration of impaired performance of an episodic-like task (OPC) in Fmr1 KO rats, indicate that transgenic rats will complement existing mouse models, providing valuable insights into the effects of FMRP loss on cognitive function.

Disclosures: **A. Asiminas:** None. **S.M. Till:** None. **A.W.A. Burt:** None. **E.R. Cox:** None. **R.G.M. Morris:** None. **S. Chattarji:** None. **D.J. Wyllie:** None. **P.C. Kind:** None. **E.R. Wood:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.16/L11

Topic: C.06. Developmental Disorders

Support: NSERC

Fondation Jerome Lejeune

NSERC - Vanier

Title: Neural precursor cells are arrested in a quiescent state in the Fragile X mouse model

Authors: ***M. SOURIAL**, E. L. TAYLOR, K. PATEL, L. C. DOERING;
McMaster Univ., Hamilton, ON, Canada

Abstract: Fragile X Syndrome (FXS) is the leading monogenic cause of autism and intellectual impairment, resulting from the lack or absence of the Fragile X mental retardation protein (FMRP). Interestingly, FMRP expression was found in astrocytes of the developing mouse brain, where wild type (WT) astrocytes rescued the abnormal morphology of Fragile X (FX) neurons in astrocyte-neuron co-cultures (Jacobs & Doering, *J Neurosci* ***30***, 4508-4514). This suggests a role for astrocytes in FXS. In addition, FMRP is implicated in neural precursor cell (NPC) biology, which comprises neural stem and progenitor cell populations. Tight regulation of NPCs is crucial for the proper formation and maintenance of neural networks. The purpose of this study was to assess the expression of early lineage cell markers in WT and FX mouse neural precursor cell

cultures, as well as examine the effect of astrocyte secreted factors on the generation of neural stem and progenitor cells. NPCs from postnatal day 1 (P1) FX and WT mouse hippocampi were grown *in vitro* as neurospheres for 4, 5, and/or 8 days. Immunocytochemistry was performed on neurospheres targeting Ki67, Nestin, and SOX2. Astrocyte conditioned media (ACM) from cortical and hippocampal WT and FX brains were added to the Neural Colony Forming Cell (NCFC) assay to examine the effect of ACM on the generation of stem and progenitor cells. In the NCFC assay, neural stem cells are distinguished from progenitor cells based on the size of the neurosphere colony. Preliminary results indicate that FX neurospheres show increased expression of SOX2 after 4 ($p<0.001$) and 8 ($p=0.0104$) days *in vitro* (DIV) and decreased expression of Ki67 after 8 DIV ($p<0.001$) compared to WT. No difference was found in Nestin expression between WT and FX. In addition, our observations suggest that astrocyte secreted factors do not have an effect on the generation of neural stem or progenitor cells in WT and FX cultures, as tested by the NCFC assay. Together these results indicate that hippocampal neural precursor cells from FX mice are arrested in a more quiescent state and do not cycle as evident by the increased SOX2 and decreased Ki67 expression compared to their WT counterparts.

Disclosures: **M. Sourial:** None. **E.L. Taylor:** None. **K. Patel:** None. **L.C. Doering:** None.

Poster

699. Fragile X Syndrome

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Topic: C.06. Developmental Disorders

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FRAXA Research Foundation

Title: Antiepileptic action of memantine in fragile x syndrome: Specific antagonism of NR2b-group I mGluR cross-talk

Authors: W. ZHAO, *R. K. WONG;
SUNY Downstate Med. Ctr., BROOKLYN, NY

Abstract: Memantine has been used clinically against a number of neurological disorders including Alzheimer's Disease. It is generally recognized that memantine exerts its clinical effectiveness via an antagonistic action on NMDA receptor. Questions persist as to how, in its

function as an NMDAR antagonist, memantine is clinically effective without deleterious side-effects commonly caused by other NMDAR antagonists such as ketamine. Our studies on memantine action on fragile X syndrome model mouse (fxs mouse) indicate specific actions of memantine that distinguishes it from broad spectrum NMDA blockers such as AP-5 and CPP that, at least in part, explain the unique therapeutic applicability of memantine. Group I mGluR (mGluR) function is amplified in fxs mice due to exaggerated receptor-elicited protein synthesis. In this background condition, our data show robust NMDAR-mGluR cross talk. Broad spectrum NMDAR receptor, CPP or AP-5, suppressed mGluR-induced neuronal hyperexcitability in young fxs mice (18-23 day-old) but facilitated mGluR-induced neuronal hyperexcitability in older fxs mice (>60 day-old). mGluR-induced hyperexcitability consisted of prolonged epileptiform discharges recorded *in vitro* in hippocampal slices and audiogenic seizure elicited *in vivo*. The data suggest that NMDARs exert bidirectional modulation of mGluR-mediated responses in fxs mice - facilitatory in the young and inhibitory in the old. Additional data show that the facilitatory action in the young is mediated by NR2B subunit of NMDAR and the inhibitory effect is mediated by subunits other than NR2B. Thus NR2B antagonists, ifenprodil or RO25-6981, exclusively suppress mGluR-mediated hyperexcitatbility in young animals but had no effect on neuronal excitability in older animals. Additional data show that the action of memantine mimics specific NR2B antagonists. Memantine (10 microM) potently suppressed epileptiform discharges in younger fxs preparations but was ineffective when applied to older fxs preparations. Our data suggest that NMDAR exerts bidirectional modulation of mGluR-elicited neuronal excitation and that memantine, via a specific antagonistic action on NR2B subunits of the NMDAR, inhibits the facilitatory action of NMDAR towards mGluR-mediated excitation. Through this action, memantine, or other NR2B blockers, may have potential therapeutic use in combat against epilepsy associated with fragile X syndrome.

Disclosures: **W. Zhao:** None. **R.K. Wong:** None.

Poster

699. Fragile X Syndrome

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NS050276 and RR017990 to T.A.N..

Title: BONLAC: A new profiling approach to decode the ASD proteome

Authors: *H. L. BOWLING[1], A. BHATTACHARYA[2], G. ZHANG[7], P. SMITH[3], K. KIRSHENBAUM[4], M. V. CHAO[8], T. A. NEUBERT[7], C. VOGEL[5], E. KLANN[6]; [2]Ctr. for Neural Sci., [1]New York Univ., NEW YORK, NY; [3]Dept. of Chem., New York Univ., New York, NY; [4]Dept. of Chem., [5]Dept. of Biol. Ctr. for Genomics and Systems Biol., [6]Ctr. for Neural Sci., New York Univ., New York, NY, NY; [7]Dept. of Biochem. and Mol. Pharmacol., Skirball Inst. of Biomolecular Medicine, NYU Sch. of Med., New York, NY, NY; [8]Mol. Neurobio., Skirball Inst. of Biomolecular Med., New York, NY, NY

Abstract: Autism spectrum disorder (ASD) affects 1 in 68 children in the U.S. Mutations in genes that cause certain syndromic ASDs, such as fragile X syndrome (FXS) impact regulation of protein synthesis due to the loss of translational regulator fragile x mental retardation protein (FMRP). In mouse models of these ASDs, this results in dysregulated protein synthesis, which alters protein expression and ultimately, neuronal communication and behavior. Thus, a direct comparison of proteins that are synthesized and expressed inappropriately in the brains of these ASD model mice to their wild-type littermates could yield important clues in the pathophysiology of ASD. Previous protein expression studies in these models have used whole proteome measurements, which likely masks altered de novo protein expression in circuits that underlie autistic behaviors. Thus, there is a critical need to assess protein expression profiles for ASD models utilizing a single platform that will yield circuit-specific results. To address this gap, we have developed a combinatorial method that we term BONLAC (BONCAT-SILAC), which combines two established protein detection and profiling techniques, to assess de novo protein synthesis and altered protein expression in intact circuits in brain slices. Newly synthesized proteins in these slices are dually-labeled, enriched using the NCAA tag, and then detected by mass spectrometry based on the SILAC tags. We compared the de novo proteome of FXS and WT mouse acute hippocampal slices using this method. We identified over 1500 proteins that were newly synthesized across 3 independent replicates. To determine which proteins were the most consistently altered with ASD, we developed a rank-order statistics-based analysis and performed Gene Ontology and functional clustering analyses. There was enrichment of synaptic and cytoskeletal proteins in these results, and we validated some of these candidates.. Our results contrast previously published screens that measured ribosome occupancy on FMRP target mRNAs, as not all targets were over-expressed as peptides. We hope that this method of measuring proteomic outcomes in intact tissues will uncover the precise translation landscape in fragile X syndrome and other related autistic disorders.

Disclosures: **H.L. Bowling:** None. **A. Bhattacharya:** None. **G. Zhang:** None. **P. Smith:** None. **K. Kirshenbaum:** None. **M.V. Chao:** None. **T.A. Neubert:** None. **C. Vogel:** None. **E. Klann:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.19/M2

Topic: C.06. Developmental Disorders

Title: Acamprosate treatment in a Fragile X Syndrome mouse model

Authors: *T. L. SCHAEFER[1], A. A. ASHWORTH[1], M. H. DAVENPORT[1], A. LANG[1], K. CAMPBELL[2], G. MOLINARO[3], K. M. HUBER[3], C. A. ERICKSON[1];
[1]Div. of Psychiatry, [2]Div. of Developmental Biol., Cincinnati Children's Hosp. Med. Ctr., CINCINNATI, OH; [3]Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Fragile X Syndrome (FXS) occurs as a result of a silenced fragile X mental retardation 1 gene (Fmr1) and subsequent loss of fragile X mental retardation protein (FMRP) expression. FMRP is an mRNA binding protein and plays a critical role at synapses to regulate activity dependent protein translation. Loss of FMRP alters excitatory/inhibitory signaling balance. Decreases in gamma-aminobutyric acid (GABA)(A) receptor expression and increased group 1 metabotropic glutamate receptor (mGluR) mediated events in humans with FXS as well as in mouse and *drosophila* models of the disorder have been reported. Acamprosate, a drug FDA approved for relapse prevention in the treatment of alcohol dependence in adults, is a novel agent with multiple mechanisms of action, including modification of glutamate, GABA, and dopamine (DA) neurotransmission. In regards to neuroreceptor regulation, acamprosate has been demonstrated to bind at a spermidine-sensitive site at the N-methyl-D-aspartate (NMDA) glutamate receptor, demonstrates properties consistent with mGluR5 antagonism and GABA(A) agonism, and modulates DA release via glycine and nicotinic acetylcholine receptors. Open-label human study of acamprosate in FXS has shown benefits in the Clinical Global Impressions Improvement scale with improvements in social behavior and inattention/hyperactivity noted. The purpose of this study was to determine if acamprosate improves behavior, electrophysiology, morphology, and molecular dysfunction in a mouse model of FXS. Adult male Fmr1 knock-out (KO) mice and their wild-type (WT) littermates were treated for ~30 days with 300 mg/kg acamprosate or saline (SAL). Mice were tested in several behavior paradigms beginning on day 11 of treatment. Baseline deficits in Fmr1 KO mice treated with SAL and improvements in Fmr1 KO mice treated with acamprosate were noted in acoustic startle response, UP state duration, and dendritic spine morphology in the somatosensory cortex compared to WT SAL-treated mice. Changes in molecular markers including extracellular signal-regulated kinase (ERK) will be

discussed. These data indicate that acamprosate may improve selective endophenotypes associated with FXS.

Disclosures: **T.L. Schaefer:** 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.; Confluence Pharmaceuticals LLC. **A.A. Ashworth:** None. **M.H. Davenport:** None. **A. Lang:** None. **K. Campbell:** None. **G. Molinaro:** None. **K.M. Huber:** None. **C.A. Erickson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Confluence Pharmaceuticals LLC. F. Consulting Fees (e.g., advisory boards); Confluence Pharmaceuticals LLC.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.20/M3

Topic: C.06. Developmental Disorders

Support: NIH Grant R01 NS079775

Title: Inducible mouse model of the fragile x premutation and fragile-x-associated tremor/ataxia syndrome (fxtas)

Authors: *J. '. KEITER[1], E. T. DOISY[1], R. WILLEMSEN[2], S. C. NOCTOR[1], R. F. BERMAN[1];
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Abstract: Fragile X-associated tremor/ataxia syndrome (FXTAS) is an adult-onset neurodegenerative disorder with symptoms that include intention tremors, cerebellar ataxia, lower extremity neuropathy, and cognitive decline. FXTAS develops in approximately 40% of males over the age of 50 and 8% of females over the age of 40 who carry a CGG trinucleotide repeat expansion of between 55-200 repeats in the 5'-UTR of FMR1. This premutation expansion causes a 2-3 fold increase in FMR1 mRNA and a 25-30% decrease in the FMR1 gene product, FMRP. Principal neuropathologies of FXTAS include mild brain atrophy, white matter lesions and ubiquitin-positive intranuclear inclusions in both neurons and astrocytes. How the premutation leads to disease is unknown. However, a "toxic gain of function" mechanism for

FXTAS pathology has been suggested whereby elevated FMR1 mRNA sequesters RNA binding proteins required for normal cell function. Previous research has relied on a CGG knock-in (KI) mouse model of the premutation and FXTAS, and these mice show much of the pathology seen in human disease. For the present study a doxycycline (Dox)-inducible mouse model was developed to determine whether pathology in neurons was necessary and sufficient for full expression of brain pathology. These mice were made by crossing a CaMKIIα-rtTA mouse line with a Tet-O-CGG98-GFP mouse line. The offspring ectopically express the CGG98 repeat expansion and the GFP reporter when exposed to Dox in their drinking water. As predicted, these transgenic animals show widespread expression of the CGG repeat, assessed via the GFP reporter, in the hippocampus, amygdala, neocortex and striatum. Dox-induced expression of the CGG98 repeat also resulted in formation of ubiquitin-positive intranuclear inclusions that were restricted to neurons, were seen in several brain areas, and were most prominent in the hippocampus. Mouse models have already provided important insights into the pathology in FXTAS, and these new inducible models should help to define the relative contribution of pathology in neurons and astrocytes to the full expression of disease, as well as to establish critical periods for disease and possible reversibility of pathology when expression of the CGG repeat expansion is halted.

Disclosures: J.'. Keiter: None. **E.T. Doisy:** None. **R. Willemsen:** None. **S.C. Noctor:** None. **R.F. Berman:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.21/M4

Topic: C.06. Developmental Disorders

Support: SAGE Therapeutics Research Contract

Title: A novel $GABA_A$ receptor positive allosteric modulator preferentially enhances extrasynaptic $GABA_A$ receptors and rescues deficient tonic GABAergic currents in a mouse model of Fragile-X Syndrome

Authors: *B. S. MARTIN[1], J. CORBIN[1], M. M. HUNTSMAN[2], G. MARTINEZ-BOTELLA[3], C. M. LOYA[3], F. G. SALITURO[3], A. J. ROBICHAUD[3], J. J. DOHERTY[3], M. A. ACKLEY[3]; [1]Ctr. for Neurosci Res., Children's Natl. Med. Ctr., WASHINGTON, DC; [2]Univ. of Colorado, Aurora, CO; [3]SAGE Therapeut., Cambridge, MA

Abstract: An increase in the ratio of excitatory to inhibitory (E/I) neurotransmission is a commonly implicated abnormality in many disorders of the central nervous system including autism spectrum disorders (ASDs). Tonic GABAergic transmission provided by peri- and extra-synaptic $GABA_A$ receptors can powerfully control neuronal excitability and plasticity. Extra-synaptic $GABA_A$ receptors therefore represent a potential therapeutic target to normalize hyperexcitable networks in these disorders. We have developed a novel positive allosteric modulator of $GABA_A$ receptors, SGE-872, based on endogenous neurosteroid progesterone metabolites such as allopregnanolone. Here we show that both allopregnanolone and SGE-872 enhanced tonic currents recorded from principal excitatory neurons within slices of the basolateral amygdala (BLA). At 100nM and 1μM, SGE-872 enhanced tonic currents without modulating synaptic currents, consistent with potent effects at extra-synaptic $GABA_A$ receptors. At 10μM, SGE-872 enhanced the weighted decay of spontaneous inhibitory synaptic currents. In a single gene model of autism spectrum disorder, the *Fmr1* knock-out (KO) mouse model of Fragile-X Syndrome, allopregnanolone and SGE-872 rescued a tonic GABAergic transmission deficit in principal excitatory neurons in the BLA. This deficit may underlie the heightened anxiety and social withdrawal that is present in the *Fmr1* KO mouse. Therefore neuroactive steroids such as allopregnanolone and SGE-872 may represent useful therapeutics for autism spectrum and related disorders involving hyperexcitability of neuronal networks.

Disclosures: **B.S. Martin:** A. Employment/Salary (full or part-time):; SAGE Therapeutics. 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.; SAGE Therapeutics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); SAGE Therapeutics. F. Consulting Fees (e.g., advisory boards); SAGE Therapeutics. **J. Corbin:** 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.; SAGE Therapeutics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); SAGE Therapeutics. F. Consulting Fees (e.g., advisory boards); SAGE Therapeutics. **M.M. Huntsman:** F. Consulting Fees (e.g., advisory boards); SAGE Therapeutics. **G. Martinez-Botella:** A. Employment/Salary (full or part-time):; SAGE Therapeutics. **C.M. Loya:** A. Employment/Salary (full or part-time):; SAGE Therapeutics. **F.G. Salituro:** A. Employment/Salary (full or part-time):; SAGE Therapeutics. **A.J. Robichaud:** A. Employment/Salary (full or part-time):; SAGE Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; SAGE Therapeutics. **M.A. Ackley:** A. Employment/Salary (full or part-time):; SAGE Therapeutics.

Poster

699. Fragile X Syndrome

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 699.22/M5

Topic: C.06. Developmental Disorders

Support: NIH/NINDS Grant R01NS053719

FRAXA Postdocotoral Fellowship Grant

Title: Mechanisms of cortical inhibitory plasticity in Fragile X Syndrome

Authors: *C. A. CEA-DEL RIO[1], M. M. HUNTSMAN[2];
[1]Sch. of Pharm., [2]Skaggs Sch. of Pharm. and Pharmaceut. Sciences, and Sch. of Med., Univ. of Colorado, Anschutz Med. Campus, Denver, CO

Abstract: Fragile-X Syndrome (FXS) patients exhibit behavioral phenotypes reflective of hyperexcitable circuitry across different brain regions including the cerebral cortex. A prominent phenotype is a hypersensitivity to sensory stimuli, with most patients find smooth surfaces to be irritating and sometimes painful to the touch, while others exhibit sensory "defensiveness" (Hagerman et al., 2009). Thus, both somatic sensation and cognitive interpretation of somatic activity are clearly disrupted in this disorder making the somatosensory cortex a highly relevant brain region to study FXS. While alterations in excitatory synapse function and plasticity are well established in Fmr1KO mouse models, recent studies now identify prominent defects in cortical inhibitory neurotransmission and suggest sensory hypersensitivity at least partially stems from alterations in interneuron function (Paluszkiewicz et al., 2011; Gibson et al., 2008). In this study, we hypothesize that inhibitory short and long term synaptic plasticity are disrupted by specific interneuron-drive dysfunction. To answer this hypothesis we performed whole cell patch clamp recordings on identified low threshold spiking (LTS) interneurons and pyramidal cells from control and the Fmr1KO mouse model of FXS. In these experiments we studied long term depression of the inhibition (LTDi), depolarization-induced suppression of the inhibition (DSI) and slow-self inhibition (SSI), three phenomena known to be mediated by inhibitory interneurons. Our results show that pyramidal cells from the layer II/III of somatosensory cortex of wild type mice undergo LTDi when layer IV is stimulated at HFS. Also, pyramidal cells from control animals express DSI when cells were depolarized to 0mv for 5 seconds. In contrast, pyramidal cells from the Fmr1KO mice failed to elicit LTDi, and DSI was drastically reduced. In addition, we tested whether LTS interneurons elicited SSI, an auto-inhibitory mechanism that operates in response to high neuronal activity. When LTS interneurons of wild type mice were tested, SSI was induced by a protocol of high frequency action potential (AP) activity. However, in LTS interneurons from Fmr1KOs, SSI was completed abolished. These data indicate that the inhibitory drive of the somatosensory cortex is altered. Taken together with previous reports

showing a failure in the enzymatic complex that synthesizes endocannabinoids (eCB) in Fmr1KO mice, these data further suggest that inhibitory synaptic plasticity in the somatosensory cortex is dependent on eCB mechanisms and their failure compromise the normal inhibitory control of the network.

Disclosures: C.A. Cea-Del Rio: None. **M.M. Huntsman:** None.

Poster

699. Fragile X Syndrome

Location: Halls A-C

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Program#/Poster#: 699.23/M6

Topic: C.06. Developmental Disorders

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Fondation Jérôme LeJeune

Title: Rescue of synaptic and spine phenotypes in fragile X mice with astrocyte-secreted thrombospondin-1

Authors: *C. CHENG, B. MISTRY, L. C. DOERING;
McMaster Univ., Hamilton, ON, Canada

Abstract: Astrocytes are integral for the proper formation, growth and maintenance of neurons and synaptic connections in the central nervous system. Astrocytes regulate synapse formation and integrity, and spine dynamics by releasing synaptogenic molecules in the form of soluble factors. Specifically, the astrocyte-secreted matricellular protein thrombospondin-1 (TSP-1) has been highly implicated in regulating neuronal synaptogenesis. Previously, we have shown that astrocytes can prevent the abnormal dendrite morphology and the dysregulated synapses that characterize fragile X syndrome (FXS) (Jacobs & Doering, 2010. *J Neurosci* **30**: 4508-14); the most common inherited form of autism. While we have identified that astrocytes affect synapse development *in vitro*, the role of astrocyte-based factors has not been extensively studied. Utilizing a FXS knockout mouse model and an astrocyte-neuron, non-contact co-culture system, we investigated the contributions of soluble TSP-1 in synapse development. ELISA was used to measure intracellular and secreted TSP-1 levels. We determined that TSP-1 levels in cellular lysates and astrocyte-conditioned media were markedly reduced in FXS astrocyte cultures. With antibodies directed to synaptophysin and PSD-95, we quantified the number of developing

excitatory synapses on the dendrites of hippocampal neurons. Excitatory synapse formation, measured by pre- and post-synaptic clusters, was restored when FXS neurons were grown in the presence of a normal astrocyte feeder layer. Additionally, the neurons displayed a morphological reduction in immature spine subtypes and dendritic spine length. Similarly, when FXS neurons were treated with TSP-1, astrocyte-mediated spine and synaptic alterations were restored to that of their normal counterparts. Together, these results identify astrocyte-derived TSP-1 as a strong promoter of neuronal development. Defects in astrocyte function and secreted molecules during early development may contribute to FXS synaptic and spine pathology. This study provides a strategy for the exploration of astrocyte-based therapies to correct abnormal patterns of development in FXS and autism related disorders.

Disclosures: C. Cheng: None. **B. Mistry:** None. **L.C. Doering:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 700.01/M7

Topic: C.06. Developmental Disorders

Support: Brain Stimulation and Monitoring Tools - Ministry of Industry, Trade and Labor

Title: Abnormal dlpfc activity in adult adhd patients: A tms-eeg study

Authors: U. ALYAGON, A. LAZAROVITS, D. COHEN, H. SHAHAR, *A. ZANGEN; Ben-Gurion Univ., Beer-Sheva, Israel

Abstract: The dorso-lateral prefrontal cortex (DLPFC) is a key area in executive regulation of cognitive functioning. Changes in activity level of DLPFC are associated with abnormal conditions like attention deficit hyperactivity disorder. The current study investigated differences in cortical activity of the right dorso-lateral DLPFC between adult ADHD patients and healthy subjects using a combined TMS-EEG protocol and a cognitive task that is known to recruit this specific area. Participants were stimulated with single and paired magnetic pulses (100 ms interval) targeted to the right DLPFC using a figure of 8 coil. In addition, they underwent a Stop Signal task that asses their ability to withhold a planned response. In the Stop Signal task, ADHD patients demonstrated longer stop signal reaction times (SSRTs) and higher rates of errors. Furthermore, N200 and P300 ERP components in response to the Stop Signal had lower amplitudes in the ADHD group. Specifically, N200 differences were marked above right pre-

frontal electrodes. Marked differences between the groups were also demonstrated in response to TMS stimulation. Single pulses produced a sustained (up to 450 ms post stimulation) local decline in power above the stimulation area which was significantly lower in the ADHD compared to the healthy participants group. In addition a clear long intra-cortical inhibition (i.e. lower response to the paired compared to the single pulse) was demonstrated but no difference was found between the groups. Taken together, the results are in accordance with prior studies and suggest hypo - activity of the right pre-frontal hemisphere in ADHD. Both measurements - TMS-EEG response and Stop Signal ERP may serve as bio markers to quantify effectiveness in future clinical trials for treatment of ADHD.

Disclosures: U. Alyagon: None. **A. Lazarovits:** None. **D. Cohen:** None. **H. Shahar:** None. **A. Zangen:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 700.02/M8

Topic: C.06. Developmental Disorders

Title: Commonalities in prefrontal cortical gene expression profiles between adolescent SHR and Wistar rats which showed "impulsive-like" behavior

Authors: *I. I. DELA PEÑA[1], I. DELA PENA[1], J. DELA PENA[1], D. HAN[2], B. KIM[3], M. NOH[4], C. SHIN[5], J. CHEONG[1];
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Abstract: Impulsivity is defined as the predisposition for premature, poorly planned, unduly risky or inappropriate actions and is associated with a number of neuropsychiatric co-morbidities, including attention deficit hyperactivity disorder (ADHD). There has been much interest in identifying the neurobiological mechanisms of impulsivity, and also the genetic and environmental influences that regulate the expression of this behavior. In this study, we identified commonalities in prefrontal cortical gene expression profiles between adolescent spontaneously hypertensive rats (SHR), animal model of ADHD, and Wistar rats which showed "impulsive-like" behavior in a delayed-reinforcement paradigm, during which animals had to

choose between relatively small but immediately available rewards vs. larger but progressively delayed rewards. Microarray analyses revealed changes in expression levels of transcription factor encoding-genes as well as those involved in neuronal plasticity in the prefrontal cortices of impulsive, juvenile SHR and Wistar rats. In addition, these analyses also showed differential expression patterns of inflammation- and neuronal apoptosis-associated genes, as well as those involved in the response to stress. Further studies are warranted to determine the contribution of these altered transcripts in the mechanism of impulsivity in rodent models, and in individuals with ADHD.

Disclosures: I.I. dela Peña: None. **I. dela Pena:** None. **J. dela Pena:** None. **D. Han:** None. **B. Kim:** None. **M. Noh:** None. **C. Shin:** None. **J. Cheong:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

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Program#/Poster#: 700.03/M9

Topic: C.06. Developmental Disorders

Support: R01 MH096773

R01 MH086654

Title: Longitudinal development of functional brain networks in ADHD

Authors: *B. D. MILLS[1], L. JOHANSEN[2], O. MIRANDA-DOMINGUEZ[1], E. EARL[1], B. NAGEL[1], J. NIGG[1], D. FAIR[1];
[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Univ. of Copenhagen, Denmark, Denmark

Abstract: Longitudinal development of functional brain networks in ADHD (2,300 character limit) Brian Mills, Louise Johansen, Oscar Miranda-Dominguez, Eric Earl, Bonnie Nagel, Joel Nigg, Damien Fair The brain is a complex network of structurally and functionally interconnected regions. These regions can be broken into highly connected and central regions- the functional hubs of the brain - as well as less central but potentially equally important regions which support overall brain function. Recent findings from a cross-sectional resting state fMRI study suggest that mature hub organization is evident in late childhood and stable from adolescence to early adulthood (Hwang et al., 2013). However, the way these networks develop in ADHD is currently unknown. Therefore, the present study aimed to investigate group

differences in developmental changes in network structure in both hub and non-hub regions. Children from six to eighteen years of age (N = 100 controls, N = 90 ADHD) between the ages of 7 and 14 were assessed. Each participant had up to three MRI scans, spaced roughly one year apart. Inclusion in the study required that resting state scans have at least five minutes of motion free data after 'scrubbing' (framewise displacement < .2mm). Our parcellation scheme included 79 ROIs which were previously defined based on a community detection algorithm in a separate resting-state cohort. Two network hub measurements were used in the current study. The first was node strength, which measures the weight strength of all of a given nodes connections. Participation coefficient, the degree to which a region participates in multiple networks was also examined, which measure the relative diversity of networks to which a region belongs to. When examining group differences in the longitudinal trajectories of these graph metrics we found stable hub development, and no differences between children with ADHD and controls. In contrast, when examining the longitudinal development of non-hub regions we found a number of differences in strength and participation coefficient between groups. These distinctions included regions in the dorsal and ventral attention networks, as well as the fronto-parietal, default, and cingulo-opercular networks. Each of these findings and implications for the ADHD phenotype are discussed.

Disclosures: **B.D. Mills:** None. **L. Johansen:** None. **B. Nagel:** None. **J. Nigg:** None. **D. Fair:** None. **O. Miranda-Dominguez:** None. **E. Earl:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 700.04/M10

Topic: C.06. Developmental Disorders

Support: Division of Research, HIMFG

Title: Brain lateralization of ERP N400 in children with ADHD and their sibs and first cousins

Authors: *E. CASTRO-SIERRA[1], E. BARRAGÁN-PÉREZ[2], H. FLORES-ORDÓÑEZ[2], L. FLORES-VALADEZ[2], S. MARTÍNEZ-RODRÍGUEZ[3];
[1]Lab. of Psychoacoustics, [2]Dept of Neurol., [3]Dept of Social Work, Hosp. Infantil de Mexico FG, Mexico DF, Mexico

Abstract: Introduction. Xia et al.(1) have noticed regional hypotrophy in ventral anterior, medial dorsal and pulvinar nuclei of left thalamus of children with Attention Deficit/Hyperactivity Disorder. Efferents of pulvinar complex terminate in cortical regions in prefrontal lobes and limbic areas subserving arousal, attention, learning and memory functions and orientation to visual and auditory stimuli. Thus, structural abnormalities associated to the pulvinar complex in children with ADHD could contribute to disrupted attention. However, there may be additional brain alterations causing the cognitive problems manifested by persons with ADHD. Methods. Taking into consideration the genetic components of this disorder(2), linguistic semantic abilities were studied analyzing ERP N400 activity of 10 patients with ADHD (7M/3F; avg. age, 10.85; avg. IQ, 99) and 11 of their sibs or first cousins without overt signs of the disorder (5M/6F; avg. age, 11.12; avg. IQ, 96) using high density EEG (256 channels, EGI, Eugene, OR, USA). Pairs of words with either semantic relatedness (e.g., AUTOMOBILE vs. AMBULANCE) or lack of it (e.g., AUTOMOBILE vs. ANIMAL) appeared on a computer monitor and subjects had to respond on a keyboard whether the words were related or not while their cortical activity was being measured. Results. Data were normally distributed and evidenced increased latency of N400 in regions corresponding to left pars opercularis and pars triangularis of patients, and diminished amplitude of N400 in left-hemisphere regions corresponding to anterior cingulum ($t = 2.584$, $p = 0.029$), pars opercularis ($t = 4.646$, $p = 0.001$) and pars triangularis ($t = 5.3$, $p = 0.000$) of patients, and to anterior cingulum ($t = 2.736$, $p = 0.021$), pars opercularis ($t = -2.275$, $p = 0.046$) and pars triangularis ($t = 2.372$, $p = 0.039$) of their sibs and first cousins. Discussion. Covering either the left eye-right hemisphere or the right eye-left hemisphere visual pathway of normal subjects while testing for N400 sets in evidence a decreased amplitude of the potential in these persons' left hemisphere(3). Thus, the present data of fixed differences of N400 amplitude between hemispheres in ADHD patients and, to a lesser degree, their unaffected relatives point at a block of normal semantic transfer between hemispheres in these persons. Further studies are in progress to analyze the presence or absence of these inter-hemispheric differences in normal children of similar age without ADHD. 1. Xia S et al. Psychiat Res: Neuroimaging 2012; 204:161-167 2. Lawrence KE et al. J Am Acad Child Adolescent Psychiat 2013; 52:431-440. 3. Wlotko E, Federmeier KD. Front Psychol 2013; 4:doi: 10.3389/fpsyg.2013.00181

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Poster

700. Attention Deficit Hyperactivity Disorder

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Topic: C.06. Developmental Disorders

Support: NIMH: 1 K23 MH087831-01A1

NIH:1R01 DA020269-01

Title: Differences in white matter microstructure associated with impulsivity in bipolar and attention-deficit/hyperactivity disorders

Authors: *J. B. KING[1], D. YURGELUN-TODD[1,2], J. DIMUZIO[1], A. STOECKEL[3], M. P. LOPEZ-LARSON[1,2];
[1]The Brain Inst., [2]Psychiatry, [3]Univ. Neuropsychiatric Inst., Univ. of Utah, Salt Lake City, UT

Abstract: Overlap in symptom profiles, which include impulsivity, have been reported in youths with bipolar disorder (BP) and attention-deficit/hyperactivity disorder (ADHD). We aimed to investigate whether or not clinical measures of impulsivity would be associated with similar microstructural changes in white matter (WM) integrity in several major WM tracts in youths with BP and ADHD. Twenty-two sex-matched youths with BP (age 15.2 ± 2.0) and 18 with ADHD (age 12.6 ± 2.1) underwent diffusion tensor MR scanning on a 3T scanner. Data were processed using FSL's TBSS and mean fractional anisotropy (FA) was extracted for major WM tracts included in the JHU White-Matter Tractography Atlas. Symptoms of impulsivity were assessed using the Barratt Impulsiveness Scale (BIS). T-tests were used to evaluate between group differences in impulsivity and Pearson correlations were used to assess the relationship between mean FA and impulsivity symptoms. Tract-based voxel-wise comparisons were also conducted for BP and ADHD groups using BIS subscale and total scores as regressors. Voxels with a $p \leq 0.05$ were considered significant. No significant differences were found between groups in BIS impulsivity scores. For the BP group, negative correlations were found between BIS non-planning (BIS-NP) and FA in the left cingulum cingulate gyrus (CCG), right corticospinal tract (CST), left and right inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus (IFOF), and left uncinate fasciculus (UF). Negative correlations were also found between BIS-NP and BIS total (BIS-T) scores and FA in the left and right anterior thalamic radiations, left and right cingulate hippocampus, and left IFOF. Additionally, BIS-NP, BIS motor, and BIS-T were negatively correlated with left CST and forceps minor FA values. No significant correlations were found for the ADHD group. Tract-based voxel-wise comparisons supported all of the aforementioned correlations with additional relationships found in the forceps major, right CCG and UF, and the left and right superior longitudinal fasciculus. For the ADHD group, again no significant associations were found between FA and any of the BIS measures. We found that increased impulsivity is associated with decreased FA in several major WM tracts in youths with BP only. These findings together with the lack of an association between impulsivity symptoms and FA in ADHD suggest that different neural mechanisms

might be responsible for the impulsive behaviors expressed in these disorders. Further work examining the neurobiological circuits driving impulsivity in these disorders is needed.

Disclosures: J.B. King: None. **D. Yurgelun-Todd:** None. **J. DiMuzio:** None. **A. Stoeckel:** None. **M.P. Lopez-Larson:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

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Topic: C.06. Developmental Disorders

Support: Children's Mercy Hospital

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AG035982

HD02528

Title: Hyperactivity, gait deficits, and orolingual motor function in the Gunn rat model of neonatal jaundice

Authors: *K. STANFORD[1], J. M. SHULER[2], S. C. FOWLER[3], S. M. SHAPIRO[4], J. A. STANFORD[2];
[1]Mol. & Integrative Physiol., [2]Univ. of Kansas Med. Ctr., Kansas City, KS; [3]Univ. of Kansas, Lawrence, KS; [4]Children's Mercy Hosp., Kansas City, MO

Abstract: Neonatal jaundice occurs in a majority of live births. Little is known about the long-term consequences of sub-kernicteric neonatal jaundice, however. Recent studies have linked mild bilirubin-induced neurological dysfunction (BIND) with attention deficit-hyperactivity disorder and autism. because of its effects on the basal ganglia and cerebellum, elevated bilirubin might affect motor function associated with these nuclei. The goal of the current experiment was to assess locomotor activity, gait, and orolingual motor function across the lifespan in the Gunn rat model of BIND. Jaundiced Gunn rats (jj) are genetically deficient of UDP glucuronosyl transferase, the liver enzyme responsible for bilirubin conjugation and clearance. Compared to their non-jaundiced (Nj) littermates, jj rats exhibited hyperactivity that was present at 3 months of age and that persisted until gait deficits emerged at late middle-age ($\geq$17 months). Gait deficits

included decreased stride length, stride velocity and propulsive force in 17-20-monthold jj rats. Unlike locomotor activity, orolingual motor function did not differ between jj and Nj rats at 3-4 months. Age-related decreases in tongue motility, however, emerged at 9-10 months of age in jj rats but not until 17-20 months in Nj rats. While speculative, our results suggest that an ADHD phenotype in young Gunn rats may be attenuated by gait abnormalities due to cerebellar dysfunction in aging.

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Poster

700. Attention Deficit Hyperactivity Disorder

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Program#/Poster#: 700.07/N1

Topic: C.06. Developmental Disorders

Support: R01 MH084947

Title: Modeling differences in probabilistic learning among children with and without ADHD

Authors: *Z. SHAPIRO[1], A. WEIGARD[2], C. HUANG-POLLOCK[2];
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Abstract: Children with ADHD commonly demonstrate academic difficulties in addition to broad patterns of impairment across multiple areas of functioning. Though theoretical models of the disorder have traditionally centered on deficits relating to executive functioning, recent conceptualizations (Nigg & Casey, 2005) have suggested a role for implicit learning. According to this idea, children with ADHD are impaired in the ability to acquire or implement knowledge related to probabilistic patterns of events in their environment. Consequently, their ability to use this information in service of self-regulatory abilities is impaired, resulting in symptoms that typify ADHD. Recently, this idea has received further empirical support in a two-alternative forced choice procedure (Weigard & Huang-Pollock, 2014). The current study examined the performance of children between the ages 8-12 with (N=214) and without (N=137) ADHD on the Stop-Signal Reaction Time (SSRT) Task. Participants' responses on trials (N) in which the presented stimulus was either identical or different from that presented in the previous trial (N-1) were compared to each other, in order to assess whether learning about the probability of these

events occurring was different across groups. Inspection of performance variables on the task indicates an interactive effect of ADHD status and trial type on response time (RT). When these data were then modeled using Drift-Diffusion Modeling (Ratcliff 2002), the derived information processing parameters derived indicate that discrepancies in response times across groups and trial type may be tied to differences in respondents' boundary separation (*a*) and the starting point of the drift diffusion process (*z*). Results will be further discussed in terms of individual differences in other neurocognitive abilities across groups as well as in regard to neurobiological models of ADHD.

Disclosures: Z. Shapiro: None. **A. Weigard:** None. **C. Huang-Pollock:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

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Program#/Poster#: 700.08/N2

Topic: C.06. Developmental Disorders

Support: FAPESP (# 2011/ 08387-6)

AFIP

FAPESP

CNPq

CAPES

Title: Comparison between physical training and physical training associated with omega-3 in the attention and executive functions in ADHD children: A Preliminary Study

Authors: *V. GRASSMANN[1], M. V. C. ALVES[1], R. F. SANTOS-GALDURÓZ[2,3], J. F. GALDURÓZ[1];
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Abstract: The physical training seems to improve the cognitive functions in several ages and pathologies, including the ADHD children. Besides the physical exercise, others types of

alternative treatment have been associated with the symptoms improvements. One of them is the consumption Polyunsaturated Fatty Acids (omega-3). The aim of this study was the comparison between physical training and physical training associated with omega-3 in the attention and executive functions in ADHD children. Were selected 16 sedentary, male, ADHD children, aged between 07-14 years old, which were not taking medicines. These volunteers were randomly assigned into two groups: Physical Training (PT; N=08) and Physical Training with Omega-3 (PTO; N=08). Both groups performed a physical training (with multiple components) during three months (three times per week on alternate days). PT group received placebo (mineral oil; 2mg/day), while PTO received 2 mg/day of fish oil. Before and after the treatment period, the following evaluations were applied: VO2 peak, Coding-WISC III subtest (concentrate attention and speed process), Stroop test (selective attention, inhibit capacity and mental flexibility) and ADHD questioner based on DSM-IV (for parents). As results the PT group shows an improvement in the total symptoms of ADHD ($p \leq 0.05$), a decrease in the time to make the Stroop test (word; $p \leq 0.05$), and an increase of the number of attempt and correct responses in the Coding subtest ($p \leq 0.05$). The PTO group shows an improvement in the inattentive and total symptoms of ADHD ($p \leq 0.05$), a decrease in the time to make the Stroop test (color and color-word; $p \leq 0.05$), and an increase of number of attempt responses in the Coding subtest ($p \leq 0.05$). When the two groups were compared the TPO showed an improvement in the time to make the Stroop test (word reading and color-word) when compared with the TP ($p \leq 0.05$). Thus, we conclude that physical training can improve the executive functions of ADHD children. Furthermore, the concomitant administration of omega-3 improves these effects even more, especially in the selective attention, inhibit capacity and mental flexibility.

Disclosures: V. Grassmann: None. **M.V.C. Alves:** None. **R.F. Santos-Galduróz:** None. **J.F. Galduróz:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 700.09/N3

Topic: C.06. Developmental Disorders

Support: Pediatric Psychopharmacology Council Fund

Title: Dissociation of working memory and attention-deficit/hyperactivity disorder in the brain

Authors: *A. MATTFELD[1], S. WHITFIELD-GABRIELI[1], J. BIEDERMAN[2], T. SPENCER[2], A. BROWN[2], J. D. E. GABRIELI[1];
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Abstract: Behavioral evidence suggests that deficits in executive functions are not mechanistically linked to attention-deficit/hyperactivity disorder (ADHD). To explore the neurobiological link between diagnostic status and deficits of executive function, we examined both behavior and brain related differences during a blocked parametric n-back working memory experiment in well-characterized, longitudinally followed participants who were diagnosed with or without ADHD in childhood. Critically, a subset of participants who were diagnosed with ADHD in childhood no longer met subthreshold criteria for diagnosis as adults (remitted), while the remaining persisted into adulthood with a diagnosis of ADHD. All ADHD participants were characterized as either impaired or unimpaired relative to controls who never had ADHD on an independent neuropsychological measure of spatial working memory. Spatial working memory status (impaired versus intact) was independent of current ADHD diagnostic status (remitted versus persistent). On the scanned n-back task, impaired ADHD participants performed worse than controls and the intact ADHD participants, whose performance did not differ from one another. There was significant linearly increasing activation as a function of working memory load in dorsolateral prefrontal cortex, intraparietal sulcus, and cerebellum of both the control group and intact ADHD group, who did not differ significantly from each other. The impaired ADHD group, on the other hand, exhibited significant hypoactivation in left dorsolateral prefrontal cortex and left intraparietal sulcus relative to the other two groups. These findings provide neurobiological evidence for a dissociation between ADHD and the executive deficits that often accompany but do not define ADHD.

Disclosures: A. Mattfeld: None. **S. Whitfield-Gabrieli:** None. **J. Biederman:** 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.; APSARD, ElMindA, Hanssen, McNeil, Shire, and VayaPharma/Enzymotec. F. Consulting Fees (e.g., advisory boards); Shinogi Pharma Inc, Cipher Pharmaceuticals, Abbott, Alza, AstraZeneca, Bristol Myers, Cephalon, Eli Lilly. **T. Spencer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Provisional Number #61/233, 686. F. Consulting Fees (e.g., advisory boards); Alcobra, Shire Laboratories, Eli Lilly, Glaxo-Smith Kline, Janssen Pharmaceutical, Novartis, Cephalon, Pfizer. **A. Brown:** A. Employment/Salary (full or part-time):; Alkermes Inc.. **J.D.E. Gabrieli:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

Location: Halls A-C

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Program#/Poster#: 700.10/N4

Topic: C.06. Developmental Disorders

Support: Litwin Foundation

Title: Psychostimulant treatment duration correlates with increased brain iron levels in attention-deficit/hyperactivity disorder

Authors: *V. ADISETIYO[1], J. H. JENSEN[1], A. TABESH[1], R. L. DEARDORFF[1], K. M. GRAY[2], J. A. HELPERN[3];
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Abstract: The first-line therapy for attention-deficit/hyperactivity disorder (ADHD) is treatment with psychostimulant medication which reduces clinical symptoms in approximately 70% of all cases. Because psychostimulants act primarily by increasing synaptic dopamine (DA) levels in the striatum and basal ganglia regions, DA deficiency has been implicated in ADHD pathophysiology. Indeed, using positron emission tomography (PET), several groups detected reduced DA biomarkers in medication-naïve ADHD patients and increased DA markers in psychostimulant medicated patients. However, the radiation exposure required to measure DA biomarkers with PET precludes its widespread clinical use. To bypass this limitation, we recently examined brain iron levels in ADHD. Given that brain iron is required for DA synthesis, we hypothesized that brain iron may serve as a possible indirect biomarker of DA status in ADHD. Previously, we reported that striatal and thalamic iron levels measured with non-invasive magnetic resonance imaging (MRI) were significantly reduced in medication-naïve ADHD patients compared to controls while brain iron levels in ADHD patients with a history of psychostimulant treatment were comparable to controls. Here, utilizing the MRI methods of magnetic field correlation (MFC) imaging and the proton relaxation rate R2* to measure brain iron levels, we examined psychostimulant treatment duration and its effects on brain iron levels in a separate cohort of psychostimulant medicated ADHD patients (n = 8; ages 8-18 years). Similar to our previous findings, the medicated ADHD group had comparable MFC and R2* indices of brain iron in the globus pallidus (GP), putamen (PUT), caudate nucleus (CN) and thalamus (THL) to age-, gender- and IQ-matched controls (n = 10; all $p > 0.05$). While brain iron levels in all regions significantly increased with age in the control group ($p < 0.05$), there was a lack of significant age-related effects in the medicated ADHD group ($p > 0.05$). In contrast, longer psychostimulant treatment duration in the medicated ADHD group was significantly correlated with increased brain iron levels in the GP (MFC $p = 0.019$; R2* $p = 0.031$), PUT (R2*

p = 0.009) and CN (R2* p = 0.043) - regional targets of psychostimulants. These findings suggest that the lack of age-related brain iron increases in the medicated ADHD group may be compensated by psychostimulant medication, with longer treatment duration resulting in normalized brain iron levels comparable to controls.

Disclosures: V. Adisetiyo: None. **J.H. Jensen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder for the MFC imaging method; patent is owned by NYU. **A. Tabesh:** None. **R.L. Deardorff:** None. **K.M. Gray:** None. **J.A. Helpern:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder of the MFC imaging method; patent owned by NYU..

Poster

700. Attention Deficit Hyperactivity Disorder

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 700.11/N5

Topic: C.06. Developmental Disorders

Support: NWO BrainGain Smart Mix Program SSM06011

Title: fMRI-based neurofeedback from anterior cingulate cortex for adults with attention-deficit/hyperactivity disorder. A proof of concept study

Authors: *A. ZILVERSTAND[1], B. SORGER[1], R. GOEBEL[1,2], J. BUITELAAR[3,4]; [1]Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands; [2]Dept. of Neuroimaging and Neuromodeling, Netherlands Inst. for Neurosci. (KNAW), Amsterdam, Netherlands; [3]Dept. of Cognitive Neurosci., Donders Inst. for Brain, Cognition and Behavior, Nijmegen, Netherlands; [4]Karakter Child and Adolescent Psychiatry, Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: Attention-deficit/hyperactivity disorder (ADHD) is associated with poor cognitive control and hypofunctioning of the dorsal anterior cingulate cortex (ACC) during continuous performance, response inhibition and switching tasks. We investigated for the first time whether fMRI neurofeedback training targeted at increasing activation levels in the ACC in adults with ADHD leads to reduction of ADHD symptoms and improved cognitive functioning. An exploratory, randomized controlled treatment study with blinding of the participants was conducted. Participants with ADHD (n=7 in the active feedback condition, and n=3 in the control condition) attended four weekly training sessions, during which they performed a mental

calculation task at varying levels of difficulty, in order to learn how to up-regulate ACC activation. Prior, and after the training ADHD symptoms and cognitive functioning were assessed by neuropsychological testing. For neurofeedback participants activation levels during cognitive training runs increased over sessions, being low in the first and second session, increasing significantly during the third session in comparison to the second session ($F(1,6) = 6.6$, $p < 0.05$, $\eta p2 = 0.52$), and remaining high during the fourth session. The neurofeedback group did not show a significant reduction of ADHD symptoms, but improved considerably and significantly on measures of attentional control (missed targets: pre-test = 2.8 z-scores below norm, post-test = 1.1 z-scores below norm, $F(1,6) = 6.0$, $p = 0.05$, $\eta p2 = 0.49$) and working memory (accuracy: pre-test = 67%, post-test = 76%, $F(1,6) = 7.7$, $p < 0.05$, $\eta p2 = 0.56$). As measurements are still ongoing, between-group statistical comparisons were not performed due to the unbalanced and small sample size. Individual modulation performance in the neurofeedback group was highly correlated with ability to sustain attention (0.80, $p = 0.03$) and working memory capacity (0.85, $p = 0.02$), but modulation success was not highly correlated with treatment change (0.42 - 0.58, not significant). The contribution of the provided neurofeedback information to treatment change could therefore not be finally evaluated.

Disclosures: **A. Zilverstand:** None. **B. Sorger:** None. **R. Goebel:** None. **J. Buitelaar:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: NIMH Grant MH065395-01A2

Title: Effect of methylphenidate on inhibitory control depends upon DAT1 genotype and baseline executive functioning in childhood ADHD

Authors: ***A. PEARCE**[1], L. KENEALY[2], W. GAILLARD[2], M. STEIN[3], E. COOK[3], C. VAIDYA[1,2];
[1]Georgetown Univ., Washington, DC; [2]Children's Natl. Med. Ctr., Washington, DC; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Molecular genetic studies show that inheritance of two copies of the 10-repeat allele (10/10) of the dopamine transporter gene (*DAT1*) is associated with susceptibility to Attention

Deficit Hyperactivity Disorder (ADHD). Blocking the dopamine transporter protein (DAT) by methylphenidate (MPH), increases dopamine signaling in the striatum by reducing dopamine reuptake. MPH clinical response varies across children and sources of variability are not well understood. We examined the extent to which DAT1, baseline (i.e., off-MPH) executive functioning and ADHD symptoms relate to MPH-induced improvement in inhibitory control. Forty-three 7-13 year old children with Combined-type ADHD (10/10, n=21; 9/10, n=22) were tested once on their prescribed dose of MPH and once 24 hours after their last dose, on a Go-NoGo task on which they responded to all letters (Go trials) except the letter "X" (NoGo trial). Inhibitory load varied across two runs, with 12.5% NoGo trials for the high-load and 25% NoGo trials for low-load. Executive functioning was measured by parent-report on the Behavioral Rating Inventory of Executive Functioning (BRIEF) and ADHD symptoms by the ADHD Rating Scale. A DAT1 x Load x MPH ANOVA showed that response inhibition (i.e., NoGo errors) was better on-MPH than off-MPH (main effect of MPH, $p < .001$) regardless of genotype and loads Regardless of MPH, response inhibition was worse in 10/10 than 9/10 children during high but not low load (DAT1 x Load $p<0.001$). Associations between MPH response (off-MPH minus on-MPH NoGo errors) and the BRIEF and ADHD Rating scale were analyzed using separate linear regression models for each load and scale, with DAT1 as an indicator variable and baseline errors and age as covariates of no interest. MPH response was related to BRIEF scores in 9/10 but not 10/10 children during low ($p=.03$) but not high inhibitory load such that during low load, 9/10 children with worse baseline executive function improved more on-MPH whereas those with better executive function performed worse on-MPH. MPH response was homogeneous during high load, with most children showing improvement. MPH response was not associated with ADHD symptoms. Thus, overall inhibitory performance depended on DAT1 genotype such that a condition that was more taxing of dopaminergic function (high load) was more deleterious for the 10/10 DAT1 genotype (10/10). For the 9/10 genotype, heterogeneity of MPH response was explained by baseline executive function during lower inhibitory demands. Thus, inhibitory demands of the task at hand, baseline executive function, and genotype status contribute to variability in MPH response in ADHD.

Disclosures: A. Pearce: None. **L. Kenealy:** None. **W. Gaillard:** None. **M. Stein:** None. **E. Cook:** None. **C. Vaidya:** None.

Poster

700. Attention Deficit Hyperactivity Disorder

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Topic: C.06. Developmental Disorders

Support: GR-2010-2315883 Ministero Salute Italy

Title: Genetic-driven partial reduction of the dopamine transporter (DAT) in mice produces ADHD but not schizophrenia-relevant behavioral phenotypes

Authors: *M. MEREU[1], G. CONTARINI[2], R. BRUNORO[2], F. PAPALEO[3];
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Abstract: Attention deficit hyperactivity disorder (ADHD) and schizophrenia are psychiatric disorders highly heritable with a strong genetic component. The neuropathophysiology of both these diseases share alterations in the dopaminergic system, however, it is not yet clear how genetic variations influencing the dopaminergic system might differentially give rise to ADHD or schizophrenia. Indeed, while sharing common features (e.g. some cognitive deficits), these two diseases have distinctive peculiar phenotypes: ADHD behavioral alterations are more pronounced earlier in life while the opposite is true for schizophrenia, hyperactivity is more evident in ADHD while sensorimotor gating deficits are more consistently found in schizophrenia, psychostimulants ameliorate the symptomatology in ADHD while exacerbate it in schizophrenia. The dopamine transporter (DAT) is the major player in the clearance of extracellular dopamine in the striatum and has been suggested as a potential susceptibility gene involved in both ADHD and schizophrenia. Mice lacking the DAT (-/-) have been extensively studied. However, DAT-/- mice present extreme phenotypes more relevant to the DAT deficiency syndrome (early Parkinson's disease). To better mimic human conditions possibly relevant to ADHD and/or schizophrenia, here we used mice carrying a partial reduction of DAT (i.e. DAT+/-) to investigate how this genetic mutation may contribute to the development of these two psychiatric disorders. These mice were then assessed for behavioral phenotypes relevant to ADHD and/or schizophrenia neuropathology from infancy to adulthood, using different behavioral tasks such as Prepulse Inhibition, Locomotor Activity and Temporal Order Object Recognition. DAT +/- mice exhibited an early appearance of increased levels of locomotor activity that could be reverted by amphetamine treatments. In contrast, sensorimotor gating abilities measured with prepulse inhibition of acoustic stimuli were normal throughout the development. Our findings highlight how DAT genetic mutations might be more relevant to ADHD rather than schizophrenia and establish the DAT+/- mutant mice as a valid experimental model of ADHD.

Disclosures: **M. Mereu:** None. **G. Contarini:** None. **R. Brunoro:** None. **F. Papaleo:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

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Topic: C.08. Ischemia

Support: NIH Grant 1R21NS078774-01A1

AHA Grant 12POST12050706

Title: Neuroprotection by neuregulin-1 after ischemia by activating ErbB4 in parvalbumin-postive interneurons

Authors: *Y. CHEN[1], Q. ZHANG[2], D. BRANN[2], L. MEI[2];
[1]Georgia Regents Univ., AUGUSTA, GA; [2]Georgia Regents Univ., Augusta, GA

Abstract: Stroke is one of leading causes of death and adult disability in the world. In stroke, ischemic insult leads to death of principal neurons and thus impairs brain function. NRG1 is a trophic factor that has been implicated in neural development, neurotransmission, and synaptic plasticity. Recent evidence indicates that NRG1 may play an important in neuronal survival in stroke. However, mechanisms of this effect remain poorly understood. We found that excitatory neurons were more vulnerable in PV-ErbB4-/- mice where ErbB4 was specifically mutated in parvalbumin (PV)-positive interneurons, after global cerebral ischemia/ reperfusion (GCI/R). Relative resistance of PV-positive interneurons to transient ischemia was decreased and die in advance of pyramidal neurons in PV-ErbB4-/- mice, comparing with control littermates. GABAergic inhibiton onto the PV-postive interneurons decreased and excitability of FS-spiking interneurons increased in PV-ErbB4-/- mice 24 hours after GCI/R, but not in control littermates. These results indicate a critical role of ErbB4 in PV-positive interneurons in regulating pyramidal neuron apoptosis after ischemia.

Disclosures: Y. Chen: None. **Q. Zhang:** None. **D. Brann:** None. **L. Mei:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

Location: Halls A-C

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Program#/Poster#: 701.02/N9

Topic: B.04. Ion Channels

Title: Cerebral venous potassium efflux during spreading depression

Authors: *J. L. SEIDEL[1], F. BLASI[1], D. VON BORNSTADT[1,2], C. AYATA[1,3];
[1]Radiology, Stroke and Neurovascular Regulation Lab., Massachusetts Gen. Hospital, Harvard Med. Sch., Charlestown, MA; [2]Neurol., Charité-Universitätsmedizin, Berlin, Germany; [3]Neurology, Stroke Service and Neurosci. Intensive Care Unit, Massachusetts Gen. Hosp., Boston, MA

Abstract: Objectives: Spreading depression (SD) is a wave of depolarization implicated in the pathophysiology of brain injury and migraine aura. Massive K+ efflux significantly increases extracellular potassium ([K+]e) during SD. Clearance of [K+]e is believed to be via astrocytic spatial buffering and reuptake by the Na+/K+ ATPase. Astrocytes are ideally positioned to siphon [K+]e to the vasculature from the extracellular space. However, it is unknown whether vascular clearance contributes to the restoration of [K+]e after SD. To test this, we measured [K+]e in cerebral venous blood during SD under normoxic and ischemic conditions. Methods: Male mice (C57BL/6) were anesthetized (isoflurane 2.5% induction, 1.5% maintenance, in 70% N2O/30% O2). Arterial blood pressure, pH, pO2 and pCO2 were monitored. Animals were placed in a stereotaxic frame, and two burr holes drilled under saline cooling at (mm from bregma): (i) A 1.5, L 2.0 (1 mm diameter; KCl application) and (ii) P 1.5, L 2.0 (2-3 mm diameter; DC and K+ electrodes). Cortical and venous [K+]e were measured using double barreled ion-sensitive electrodes. Venous recordings were made from pial veins (~20 μm in diameter). Extracellular (DC) potential was recorded using a glass electrode (~300 μm deep) adjacent to the K+ electrode. SD was induced by topical application of KCl solution (300 mM). Transient focal ischemia was induced by distal middle cerebral artery occlusion (dMCAo), and periinfarct depolarizations (PID) were monitored for 2 h. Results: SD resulted in a large transient increase in cortical [K+]e (36.6±3.0 mM) concurrent with the characteristic negative DC shift. Venous [K+]e also increased during SD. The magnitude of venous [K+]e increases were significantly smaller (25.3±1.9 mM, p=.003). Additionally, the duration of recovery to baseline levels was longer for venous recordings when compared to cortical measurements (43.0±3.2 s and 31.8±1.2 s, respectively, p=.0006). Venous electrode placement was confirmed by direct KCl injections (30 mM) into the same vein upstream, as well as by induced systemic [K+]e elevations. While the amplitude of venous [K+]e responses did not differ with ischemia (33.5±5.8 mM, p=.613), the duration of the [K+]e recovery to baseline was significantly prolonged (194.5±32.5 s, p< .0001). Conclusions: These data show that elevated [K+]e during SD/PID is at least in part cleared through venous outflow. Taken together with previous data

showing total tissue K+ depletion in focal ischemic brain, our findings suggest that [K+]e may be cleared directly by the venous network, representing a paradigm shift in our understanding of [K+]e regulation during SD and injury depolarizations.

Disclosures: **J.L. Seidel:** None. **F. Blasi:** None. **D. Von Bornstadt:** None. **C. Ayata:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

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Topic: C.08. Ischemia

Support: NIH Grant NS078805

NIH Grant NS051288

Title: Spreading depression of cortical activity: Lamina specificity and role of adenosine receptors

Authors: ***B. E. LINDQUIST**, C. W. SHUTTLEWORTH;
Neurosciences, Univ. of New Mexico Sch. of Med., Albuquerque, NM

Abstract: Spreading depolarization (SD) is a propagating wave of depolarization of brain tissue, accompanied by depression of evoked and spontaneous cortical activity. Spontaneous and evoked thalamocortical and corticocortical activity are affected during this depression, though the degree and time-courses of inhibition of these circuits relative to each other have not been well described. The depression of activity accompanying SD is incompletely understood *in vivo*, but from recent work in brain slices (Neuroscience 223 (2012)365-76), mechanisms of synaptic depression may include activation of adenosine A1 receptors (A1R), in addition to voltage inactivation of voltage gated sodium channels (depolarization block). We evaluated the time-course of recovery of evoked corticocortical and thalamocortical synaptic potentials and spontaneous electrocorticogram (ECoG), and tested the effects of A1R modification on suppression. Unilateral cortical SD was induced by KCl (1M) or pin-prick in C57Bl/6 mice under isoflurane or urethane anesthesia. Transcallosal field EPSPs were evoked by constant-current pulses (70μs, 0.1Hz) delivered by a bipolar stimulating electrode, and recorded in the contralateral cortex. We observed initial depolarization block during the passage of SD. However, this refractory period was insufficient to explain the longer-lasting depression of

evoked EPSPs ($p<0.05$). The depression of spontaneous ECoG significantly outlasted both depolarization block and the depression of transcallosal fEPSPs ($p<0.0001$). By contrast, the time-course of ECoG depression was similar to the depression of thalamocortical (whisker pad) evoked potentials. ECoG depression was reduced by focal application of selective A1R antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) 30 μM ($p<0.01$) and was prolonged by adenosine deaminase inhibitor deoxycoformycin 100 μM. These findings clarify the time-course of two phases of spreading depression, the first mediated by depolarization block and the second characterized by synaptic suppression. Our results indicate that activation of A1R by endogenous adenosine accumulation following SD contributes to spreading depression of cortical activity.

Disclosures: B.E. Lindquist: None. **C.W. Shuttleworth:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

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Topic: C.08. Ischemia

Support: NIA PO1 AG022550

NIA PO1 AG027956

Title: Bacterial Infection mimic exacerbates blood-brain barrier permeability in experimental stroke via compromising mitochondrial function of brain vascular endothelial cells

Authors: *X. REN, D. N. DOLL, H. HU, S. N. SARKAR, D. D. QUINTANA, J. W. SIMPKINS;
Ctr. for Basic and Translational Stroke Research, Physiol. & Pharmacol. PO, West Virginia Univesity Robert C. Byrd Healthe Sci. Ctr., Morgantown, WV

Abstract: Stroke is the fourth leading cause of death in the U.S, with many cases leading to severe disabilities. Acute infection is a generic trigger for acute ischemic stroke in people. Statistics reports present that more than 40% of ischemic stroke patients have recent prior infections. Previous studies have demonstrated that, 3 hours prior to stroke, systemic infectious mimic with Lipopolysaccharide (LPS) profoundly impairs survival, and increases blood-brain barrier (BBB) injury and brain edema at 24 hours after experimental stroke. Using an experimental ischemic stroke model, here we also demonstrated that a low dose of LPS

(100μg/kg) prior to transient middle cerebral artery occlusion increased the permeability of BBB following 30 min occlusion and 7 hours reperfusion in mice. Further in an *in vitro* study, LPS (1μg/ml) significantly up-regulated the expression of miR-146a, a mediator of inflammation, and strongly reduced miR-34a, a regulator of cell apoptosis, in the exosome of cultured brain vascular endothelial cells. More interestingly, LPS (dose range of 0.1-100μg/ml) robustly reduced maximal respiration (30-40% compared to control) in brain vascular endothelial cells *in vitro*. These data are particularly important because they suggest a novel mitochondrial mechanism of the interactions between stroke and infections on BBB integrity.

Disclosures: X. Ren: None. **D.N. Doll:** None. **H. Hu:** None. **S.N. Sarkar:** None. **D.D. Quintana:** None. **J.W. Simpkins:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

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Topic: C.08. Ischemia

Support: NIA PO1 AG022550

NIA PO1 AG027956

Title: Dynamic blood-brain barrier openings concur with increasing of miR-let7a in murine experimental stroke

Authors: *H. HU, X. REN, S. JUN, S. N. SARKAR, D. N. DOLL, J. W. SIMPKINS; Physiolgoy & Pharmacol., West Virginia University, Ctr. For Basic and Translational Stroke Res., Morgantown, WV

Abstract: Ischemic stroke is a devastating central nervous system and cerebrovascular event marked by ischemic brain cell death and breakdown of blood-brain barrier (BBB). BBB openings to plasma proteins lead to vasogenic edema in humans and in experimental models of ischemic stroke. Regulation of early BBB openings may control brain edema, limit penumbra damage and reduce ischemic volume. It is known that non-coding microRNAs (miRNAs) regulate both biological and pathological processes, including maintenance and breakdown of BBB in stroke, by modulating the stability and/or the translational efficiency of target messenger RNAs. In our studies, dynamic BBB opening was detected using Evans Blue (EB) as a tracer and

demonstrated two peaks of active BBB openings after reperfusion following one hour of the middle cerebral artery occlusion. EB extravasation was observed at 6 hours and 72 hours post reperfusion but not at 30min, 24 hours or 48 hours reperfusion. Interestingly, early BBB openings concurred with greatly increasing of miR-let7a in the mouse serum and ischemic hemisphere at 6 hours post reperfusion compared to sham animals. Furthermore, an *in vitro* BBB opening model using stimulation by LPS also showed a significant increase of miR-let7a in cultured brain vascular endothelial cells. These novel data suggest miR-let7a is involved in early BBB opening and may be a novel therapeutic target for ischemic stroke.

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Poster

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Topic: C.08. Ischemia

Support: NIH F31 NS073149

NIH RO1 NS081055

The Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Vascular production of colony stimulating factor 1 (CSF1) modulates post-stroke neurogenesis

Authors: *A. J. BRUMM, M. MACHNICKI, G. COPPOLA, S. T. CARMICHAEL; Neurol., UCLA, Los Angeles, CA

Abstract: Ischemic stroke stimulates the proliferation of neural progenitor cells (NPCs) in the subventricular zone (SVZ) and their migration as doublecortin-positive neuroblasts to sites of ischemic damage in the brain. Using a mouse model of distal middle cerebral artery occlusion (dMCAO), we have shown that SVZ-derived neuroblasts preferentially migrate to angiogenic vasculature in peri-infarct cortex (PI ctx) and form a neurovascular niche adjacent to the stroke core. Angiogenesis causally regulates neurogenesis within this niche, but the specific angiogenic blood vessel-derived (angiocrine) factors that mediate neuroblast recruitment, survival, and differentiation have not been well described. We developed a set of candidate vessel-neuroblast

signaling interactions through cell-specific whole genome expression profiling at 7d after stroke, the peak of post-stroke angiogenesis and neurogenesis. These data identified the cytokine macrophage colony stimulating factor (M-CSF/CSF1) and its receptor (CSF1R/CD115) as a novel receptor-ligand system upregulated in stroke-responsive neuroblasts and peri-infarct angiogenic blood vessels, respectively. *In vitro* studies with adult mouse SVZ-derived neural progenitor cells (NPCs) demonstrated that exogenous CSF1 (10-100 ng/ml) increased NPC proliferation and modulated neuronal differentiation/survival. *In vivo* CSF1 gain-of-function, via hydrogel release of recombinant mouse CSF1 protein (30 ug/ml) to PI ctx starting at 3d after dMCAO, increased the number of stroke-responsive neuroblasts within PI ctx by 40.2% compared to vehicle control at 14d after MCAO. Blood vessel-specific overexpression of CSF1, via injection of an endothelial VE-cadherin promoter-driven lentivirus into PI ctx at the time of stroke, similarly increased the stroke-responsive neuroblast population within PI ctx at 14d after dMCAO (53.8% vs. empty vector). These results demonstrate that CSF1 modulates the proliferation and differentiation potential of SVZ-derived NPCs and increases peri-infarct neurogenesis after stroke, and suggests a significant role for vessel-derived CSF1 in peri-infarct niche signaling. Ongoing *in vivo* experiments are using both CSF1 overexpression and anti-CSF1 miRNA lentiviruses to investigate the role of vascular CSF1 in post-stroke neuroblast recruitment and long term survival/neuronal differentiation. These studies provide insight into an endogenous signaling system that regulates post-stroke neurogenesis, a process with demonstrated impact on functional recovery from stroke in rodent models.

Disclosures: A.J. Brumm: None. **S.T. Carmichael:** None. **M. Machnicki:** None. **G. Coppola:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 701.07/O2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cell-based immunotherapy for intracranial hemorrhage

Authors: *H. DOU[1], X. WANG[2,1], J. RODRIGUEZ[1], T. THOMAS[1], L. DO[1], L. ZOU[1]; [1]Dept. of Biomed. Sci., Texas Tech. Univ. Hlth. Sci. Ctr. (TTUHSC), El Paso, TX; [2]Anat., Weifang medical Univ., Weifang, China

Abstract: Backgroud: Stroke caused by intracranial hemorrhage (ICH) is an important clinical problem that can leave affected people with permanent neurological deficits for which no treatment is available. ICH-induced inflammation appears to be a key factor in secondary brain damage, as evidenced by a reduction in brain damage in immune-deficient animal models of central nervous system (CNS) disorders, including stroke. We have found bone marrow derived dendritic cell (DC)-like cells (BMDC) not only to regulate systemic immune responses but also to modulate multiple brain functions following brain injury. Methods: For inducing ICH, mice will be anesthetized and secured with ear bars and a mouthpiece on a stereotactic apparatus. Peripheral blood, collected from the facial vein will be injected into the left hemisphere of the brain. Mouse bone marrow cells were treated with IL4+ MCSF for 5-7 days to produce BMDC. In order to study the systemic immune response and neuroprotective activities in BMDC treated to the mouse model of ICH, splenic cells and cytokines of the innate immune activity and neuronal loss were analyzed by flow cytometry, histology, Immunofluorescence staining and RT-PCR. Results: BMDC differentiation showed over 94% of CD45+/CD11b+ and CD45+/CD80+ cells following IL4+ MCSF treatment. IL-4 and GM-CSF treatment significantly expanded the activated and matured CD86+, CD83+ and CD163 cells and decreased CD68+ inflammatory populations. CD11c+ cells remained elevated for up to 7 days. ICH-induced Iba-1+ microglia activation was correlated with neuron loss. The greatest levels of microglia were parallelled with an increase in degenerated neurons (reflected by pNF aggregation) on day 7 after ICH. Immunohistochemistry was used to examine neuroprotection of BMDC. Quantitation images revealed a significant loss of Map-2+ density and increase of Iba-1+ staining in ICH group. BMDC treatment prevented neuronal loss and exhibited greater levels of Map-2+ neuritis. The protective immune responses showed that splenic CD11b+ inflammatory populations and CD8 cytotoxic T cells were decreased in BMDC treated ICH group. With BMDC treatment, ICH mice reduced TNF-α and INF-γ expression and up-regulated IL10 and TGF-β levels in spleen. Importantly, BMDC reversed all tested cytokine levels in the brain. Conclusion: anti-inflammatory activities by exogenous regulators may be too dangerous for injury prevention and repair. BMDC based neuroprotective therapy will lead to an increase in autologous anti-inflammatory activities, a decrease in the inflammatory signaling, and restored neuronal injury.

Disclosures: H. Dou: None. **T. Thomas:** None. **J. Rodriguez:** None. **L. Do:** None. **L. Zou:** None. **X. Wang:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 701.08/O3

Topic: C.08. Ischemia

Support: NIH RO1 NR005260

Title: Role of proteolytic Isoforms of BDNF in stroke-induced depression-like symptoms and myelin changes in the corpus callosum

Authors: *T. L. BRIONES[1], J. WOODS[2], M. WADOWSKA[2];
[1]Adult Hlth. Dept., Wayne State Univ., Detroit, MI; [2]Adult Hlth., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Among the morphological and behavioral consequences of stroke are white matter structure abnormalities and depression. Despite the increasing recognition of these negative consequences post stroke, there is a lack of understanding regarding their underlying mechanisms. Here we examined the expression of the different isoforms of brain-derived neurotrophic factor (BDNF) and its corresponding receptor as the possible common mechanism that mediates post-stroke depression and myelin changes in the corpus callosum. Thirty male Wistar rats were included in the study and randomly assigned to either ischemic stroke (endothelin-1) or sham group (saline). Sensorimotor function was assessed one week after recovery from stroke and depressive phenotype was assessed three days after. Our data showed that endothelin-1 (ET-1) injection results in circumscribed infarct in the ipsilateral brain hemisphere and that this injury produced sensorimotor impairments on the contralateral limbs and depression-like symptoms. In addition, we found that ET-1 injection resulted in decreased myelin sheath thickness in the corpus callosum compared to the sham group. Decreased expression of the mature and precursor forms of BDNF while the truncated form of BDNF increased after stroke. As well, the full-length isoform of the BDNF receptor TrkB decreased but the truncated form of TrkB (trkB.t1) increased in the ET-1 group when compared to the sham animals. The increased expression of TrkB.t1 was significantly related to decreased sucrose consumption and myelin sheath thickness. These findings suggest that BNDF expression and signaling play a role in the behavioral impairment and structural changes seen following ischemic stroke.

Disclosures: **T.L. Briones:** None. **J. Woods:** None. **M. Wadowska:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 701.09/O4

Topic: C.08. Ischemia

Support: ERA-NET Neuron project REVIS

Title: Immediate changes in neuronal activity induced by stroke in cat visual cortex

Authors: *W. J. WALESZCZYK[1], P. URBAN[1], A. FOIK[1], J. JABŁONKA[1], P. MALINOWSKI[2], E. KUBLIK[1], R. KUŚ[2], M. KAMIŃSKI[2], J. ŻYGIEREWICZ[2];
[1]The Nencki Inst., Warsaw, Poland; [2]Inst. of Exptl. Physics, Fac. of Physics, Univ. of Warsaw, Warsaw, Poland

Abstract: The purpose of this study was to elucidate immediate changes in neuronal activity induced by a stroke in the cat visual cortex. Photothrombotic stroke was induced in acute experiments in cats anaesthetized with izoflurane in a gaseous mixture of nitrous oxide and oxygen. The activity of neuronal populations in the central region of the stroke, the stroke border, and healthy tissue, were monitored by recording local field potentials with single channel electrodes spaced ~1 mm apart. An additional eight-channel vertical electrode was located 4 mm from the centre of the stroke core. Signals recorded before, and up to three hours after the stroke, were analysed off-line using various methods. Power spectra were obtained with Welch and autoregressive parametric methods. Functional connectivity was determined by crosscorrelation analysis and spectral coherence. In addition, direct and indirect connections between neuronal assemblies were respectively determined by Partial Directed Coherence and Directed Transfer Function. A stroke resulted in an overall decrease in the power spectrum in the stroke affected region, but not outside this region, where an increase in the power spectrum was observed. The most pronounced changes were observed three hours after the stroke. Crosscorrelation analysis revealed a weakening of neuronal connections between the healthy tissue and the stroke region and a strengthening of local connections outside the stroke region. As expected, the stroke causes opposite effects in the power spectrum of the signal recorded inside and outside the stroke affected region, but also leads to a disturbance of connections between neuronal assemblies in the vicinity of stroke.

Disclosures: W.J. Waleszczyk: None. **P. Urban:** None. **A. Foik:** None. **J. Jabłonka:** None. **E. Kublik:** None. **P. Malinowski:** None. **R. Kuś:** None. **M. Kamiński:** None. **J. Żygierewicz:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 701.10/O5

Topic: C.08. Ischemia

Support: VR 2013-2475

Title: The immune response after hypoxia-ischemia in a mouse model of preterm brain injury

Authors: *X. WANG[1], A.-M. ALBERTSSON[2], D. BI[2], X. ZHANG[2], J. LEAVENWORTH[3], S. CARDELL[2], H. CANTOR[3], H. HAGBERG[2], C. MALLARD[2];
[1]PMC, Inst. of Neurosci. and Physiology, Univ. of Gothenburg, Gothenburg, Sweden; [2]Univ. of Gothenburg, Gothenburg, Sweden; [3]Dana-Farber Cancer Inst., Boston, MA

Abstract: Background Preterm brain injury consists of periventricular leukomalacia (white-matter injury) accompanied by elements of gray-matter injury, and these injuries are associated with cerebral palsy and cognitive impairments. Inflammation is believed to be an important contributing factor to these injuries. The aim of this study was to examine the immune response in a postnatal day (PND) 5 mouse model of preterm brain injury induced by hypoxia-ischemia (HI) that is characterized by focal white and gray-matter injury. Methods C57Bl/6 mice at PND 5 were subjected to unilateral HI induction by left carotid artery ligation and subsequent exposure to 10% O2 for 50 min, 70 min, or 80 min. At 7 days post-HI, the white/gray-matter injury was examined. The immune responses in the brain after HI were examined at different time points after HI using RT-PCR and immunohistochemical staining. Results Only HI for 70 min in PND 5 mice induced local white-matter injury with focal cortical injury and hippocampal atrophy, features that are most similar to those seen in preterm brain injury in human infants. HI for 50 min resulted in a small percentage of animals being injured, and HI for 80 min produced extensive infarction in multiple brain areas. Various immune responses, including changes in transcription factors and cytokines that are associated with a T-helper (Th)1/Th17 response, an increased number of CD4+ T-cells, and elevated levels of triggering receptor expressed on myeloid cells 2 (TREM2) and its adaptor protein DNAX activation protein of 12 kDa (DAP12) were observed using the HI 70 min preterm brain injury model. Conclusions We have established a reproducible model of HI in PND 5 mice that produces consistent local white/gray-matter brain damage that is relevant to preterm brain injury in infants. This model provides a useful tool for studying preterm brain injury. Both innate and adaptive immune responses are observed after HI, which shows a strong pro-inflammatory Th1/Th17 bias. Such findings provide a critical foundation for future studies on the mechanism of preterm brain injury and suggest that blocking the Th1/Th17 immune response might provide neuroprotection after preterm brain injury.

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Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

Location: Halls A-C

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Program#/Poster#: 701.11/O6

Topic: C.08. Ischemia

Support: YM 04 / 12

Title: Effects of oxygen glucose deprivation on polarization of rat microglia in primary culture

Authors: R. BARAKAT[1], *S. HASAN[2], Z. REDZIC[1];
[1]Physiol., Kuwait Univ., Kuwait, Kuwait; [2]Kuwait Univ., Mishrif, Kuwait

Abstract: Conflicting data exist on whether microglia-derived cytokines exert injurious or protective effects on brain cells after cerebral ischemia. Upon stimulation, resting microglia transform into M1 or M2 phenotype, which secrete cytokines that exert pro- and anti-inflammatory effects, respectively. The aim of this study was to explore the time course of secretion of these cytokines from microglia *in vitro* after oxygen and glucose deprivation (OGD). Primary cultures of rat microglia were exposed to 2h OGD, which was followed by a recovery period for up to 10 days. In some cases, M1 and M2 phenotypes were induced (in cultures that were not exposed to OGD) by interferon γ (IFNγ) / lipopolysaccharide(LPS) and interleukin(IL)-4 treatments, respectively. Concentrations of cytokines (expressed in pg/ml) that are specific for M1 phenotype (tumor necrosis factor α (TNFα), IL-12 and IL-6) or for M2 phenotype (transforming growth factor β -TGFβ) in the cell culture media were estimated by ELISA. A one-way analysis of variance, or in some cases Student's t-test, were used for statistical analysis. Data are presented as mean±SEM from four different experiments. After addition of IFNγ/LPS to the medium, concentrations of M1 phenotype-specific cytokines were significantly higher ($p<0.0001$) than in corresponding controls at 48h, while concentration of TGFβ did not differ from controls. After addition of IL-4, concentration of TGFβ in the cell culture medium was 1536±116.4 ($p<0.001$ vs. control); in contrast to this increase, concentration of IL-12 was below threshold values, while concentrations of TNFα and IL-6 were 107.5±5.4 and 320.1±41.1, respectively. Effect of the recovery period on concentrations of all four cytokines in the cell culture media was significant. Concentrations of IL-12, TNFα and IL-6 remained low at days 1 and 5 of the recovery period, but then increased, reaching 434.6±35.3, 367.1±29.9 and

853.8±114.6 after 10 days, respectively. Concentration of TGFβ in the medium collected immediately after OGD was 685.3±37.5 and then increased further to 997.6±31 at day 1. This was followed by a decrease to 362.4±35.2 and 187.3±11.3 after 5 and 10 days, respectively. In conclusion, 2h OGD protocol triggered secretion of phenotype-specific cytokines from microglia, but the time courses of release of M1-specific and M2-specific cytokines revealed different patterns: M2-specific cytokine increased immediately after OGD reaching a peak at day 1 of the recovery period and then decreased steadily towards day 10, while M1-specific cytokines remained low for the first 5 days of the recovery period and then increased several fold between days 5 and 10.

Disclosures: R. Barakat: None. **S. Hasan:** None. **Z. Redzic:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

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Title: Post-stroke administration of melatonin improves long-term outcome after focal ischemia/reperfusion (FI/R) via interleukin-4 (IL-4) dependent M2 microglial polarization

Authors: J. SUENAGA[1], X. HU[1], L. MAO[1], *J. CHEN[2,1];
[1]Neurol., Ctr. of Cerebrovascular Dis. Research, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Univ. Pittsburgh Sch. Med., PITTSBURGH, PA

Abstract: Background and Purpose; Microglia represent rational but difficult therapeutic targets for stroke due to their diverse phenotypes that play dual-faced protective (M2 phenotype) and toxic (M1 phenotype) effects. Previous study has shown that subcutaneous melatonin injection increased the level of IL-4, the best known M2 inducing cytokine, in the blood. In this study, we investigated the role of melatonin in microglia M2 polarization and its effect on long-term recovery after stroke. Methods; Focal cerebral ischemia was induced for 60 min FI/R. Animals were randomly assigned to receive either melatonin or vehicle treatment at 2h after stroke. Brains were assessed for cerebral tissue loss at 3 and 14 days of reperfusion. Neurological

performance was analyzed up to 14 days after ischemia. Markers for microglia polarization were assessed using immunofluorescent staining and RT-PCR. *In vitro* experiments using primary microglia and in-transwell microglia-neuron coculture were done to confirm the effect of melatonin on microglial inflammatory responses and its effect on microglia-potentiated neuronal injury upon OGD. Results; Melatonin significantly reduced infarct volume and attenuated sensorimotor deficits 3-14 day after FI/R. IL-4 deficiency, abolished melatonin-afforded long term protection. Melatonin-treated mice showed significantly reduced expression of inflammatory cytokine and chemokines, which is accompanied by significantly increased expression of M2 markers and decreased expression of M1 markers in microglia. In primary microglial cultures, melatonin inhibited LPS (a M1 inducer)-induced production of NO and TNFα, confirming that melatonin has direct anti-inflammatory effect on microglia. Furthermore, melatonin ameliorated the neurotoxic effect of M1 microglia on OGD neurons, and this effect was absent in IL-4 deficient microglia. Conclusions; Melatonin may represent an innovative therapeutic strategy that shifts microglia polarization toward a protective M2 phenotype in an IL-4-dependent manner and thus enhance long-term recovery after stroke.

Disclosures: **J. Suenaga:** None. **J. Chen:** None. **X. Hu:** None. **L. Mao:** None.

Poster

701. Ischemia: Cellular Mechanisms and Neuroprotection IV

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Title: Induced-pluripotent stem cell transplantation alleviates anxiety-like behavior in a focal ischemic stroke model of mice

Authors: *T. C. DEVEAU, M. CHAU, K. YUENGLING, S. P. YU, L. WEI;
Anesthesiol., Emory Univ., ATLANTA, GA

Abstract: Studies to date using induced pluripotent stem cell-derived neural progenitors (iNA-iPS-NPCs) as a stroke therapy have focused on functional recovery. Among the therapuetic potential, improvement of psychiatric dysfunction after ischemic stroke has received little attention. Post-stroke depression, for example, affects a majority of stroke patients. The symptoms associated with post-stroke depression and related mood disorders can interfere with patient recovery following stroke, and may interfere with treatments aimed at functional recovery. Stem cell therapy acts on multiple levels on multiple mechanisms, however the effects of stem cell therapy on chronic mood disorders following stroke are currently unexplored. The present study aims to examine the effects of stem cell transplantation on mood disorder symptoms following stroke. Mouse iPS cells were differentiated using standard differentiation methods in tandem with a novel rotary suspension technique we developed to improve neuronal differentiation (>80-90% NeuN positive). Semi-quantitative PCR analysis shows iPS-derived neurons also express growth factors VEGF and BDNF, demonstrating their potential not only for cell replacement but that they provide growth factors for neurovascular repair. *In vivo*, we employ a permanent distal middle cerebral artery occlusion in tandem with temporary bilateral common carotid occlusion to produce a focal ischemic insult located primarily in the rodent right sensorimotor cortex including the barrel cortex. iPS-NPCs or vehicle were administered by two transplantation routes: 1) delayed intranasal (1.5x10^6, 3 days after stroke) and 2) intracranial (4x10^5, 7 days after stroke). Anxiety-like behaviors were measured with the open-field test at 7, 14, and 21 days after stroke using the Top-Scan Behavioral Analysis system (Cleversys, Inc.). Preliminary observations reveal stroke animals displayed trends in decreased time spent in the middle of the open field and decreased crossings from the outside to the inside of the open field compared to sham controls. These data suggest that the stroke model can induce anxiety-like behaviors in rodents and may be tested for novel therapies. Following stem cell transplantation, we observe stroke animals that received either of the transplantation regiments increased time spent in the middle of the open field as well as crossings from the outside to the inside to near-sham levels. In addition to affecting sensorimotor functional recovery, these preliminary data suggest that stem cell transplantation may also improve symptoms of psychiatric disorders that are commonly observed in stroke patients.

Disclosures: T.C. Deveau: None. **M. Chau:** None. **K. Yuengling:** None. **S.P. Yu:** None. **L. Wei:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.08. Ischemia

Support: NSERC

Innovation PEI

Atlantic Innovation Fund

Title: The effects of focal prefrontal ischemic lesions on attentional set-shifting and cost-benefit decision making in the rat

Authors: *R. A. DÉZIEL, R. A. TASKER;
Univ. of Prince Edward Island, Charlottetown, PE, Canada

Abstract: Stroke is the leading cause of neurological disability worldwide, and it has been estimated that one quarter of stroke survivors experience cognitive impairments persisting for at least three months post-stroke. Many higher order cognitive deficits occur because of damage to the prefrontal cortex (PFC), which is one of the main areas of the brain responsible for executive functions (i.e functions which direct and plan goal-oriented behaviour) in animals. With the increasing prevalence of stroke, there is a growing requirement for accurate modelling of cognitive decline post-stroke. Currently, there are few rat models that examine the effects of stroke on executive function. Using bilateral micro-injections (1 μl) of the vasoconstricting peptide endothelin-1 (ET-1) into the medial PFC in male SD rats (or vehicle control, N = 11-12 per group) we produced highly localized ischemic lesions in the PFC as confirmed with histology and immunohistochemistry. The effects of these lesions on cognition were assessed using tests of attentional set-shifting and cost-benefit decision making. The combination of localized damage and behavioural tests of executive function shows potential for developing a model of post-ischemic cognitive dysfunction that could support subsequent studies of cognitive rehabilitation and neuroplasticity post-stroke.

Disclosures: R.A. Déziel: None. **R.A. Tasker:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

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Topic: C.08. Ischemia

Support: NSC 102-2410-H-431-005-MY3

NSC 101-2410-H-431-007

NSC 100-2410-H-431-003

Title: Examinations of explicit and implicit memory for an animal model of stroke hemorrhage

Authors: *B. H. HE[1], A. C. W. HUANG[1], B.-C. SHYU[2];
[1]Psychology, Fo Guang Univ., Yilan County, Taiwan; [2]Inst. of Biomed. Sciences, Academia Sinica, Taipei 11529, Taiwan

Abstract: It is well-known that stoke hemorrhage patients show many cognitive dysfunctions, including learning and memory. However, whether it can be dissociated explicit memory from implicit memory for stroke in the animal model remains unclear. The present study addressed this interesting issue. Firstly, all of rats were administrated a 0.125 U dose of collagenase type IV (volume, 0.5ul) or its vehicle into the unilateral injection to lesion the left ventrobasal complex of thalamus (VBC), and tested the withdrawal responses of left and right legs. All rats were randomly assigned into Shame and VBC groups. Later, all of rats were given taste aversion learning (implicit memory) and episodic memory (explicit memory) tests. The results indicated that VBC-lesion rats exhibited decreased withdrawal time to induce thermal hyperalgesia in right and left legs. It indicated that VBC-lesion rats induced the symptom of stroke hemorrhage. The intake volume of the saccharin solution was not affected for the VBC-lesion group when compared to that of the Shame group; indicating the VBC lesion did not affect the conditioned taste aversion. With regard to episodic memory tests, VBC-lesion rats spent less time in the habituation object. Also, VBC-lesion rats showed lesser trial numbers for searching the habituation object. It suggested that VBC-lesion rats actually impaired the performance of episodic memory test. Altogether, VBC-induced stroke hemorrhage may not affect conditioned taste aversion learning, but it decreased the episodic memory of E-maze task. The findings of dissociations of explicit memory and implicit memory should be investigated in the further studies. Keywords: stroke, stroke hemorrhage, explicit memory, implicit memory, conditioned taste aversion, episodic memory, rat

Disclosures: **B.H. He:** None. **A.C.W. Huang:** None. **B. Shyu:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.03/O11

Topic: C.08. Ischemia

Title: Application of low-frequency intracortical electrical stimulation to minimize neuronal hyperexcitability in a rat model of ischemic stroke - Preliminary findings

Authors: R. NIELSEN[1], *W. JENSEN[2];
[1]Ctr. for Sensory-Motor Interaction, Dept. of Hlth. Sci. and Technol., [2]Aalborg Univ., Aalborg, Denmark

Abstract: Introduction: Ischemic stroke occurs as a cascade of events that evolve over time and space. Due to excessive release of excitatory neurotransmitters from damaged neural structures, cells in the lesser affected regions will repeatedly depolarize and in the end become necrotic. Electrical stimulation of ischemic tissue has been shown to reduce necrosis and intracortical (IC) recording via microelectrode arrays have given new insight into the neural processes that occur after stroke. The objective of the present study was to establish a rat model of ischemic stroke applying low-frequency IC stimulation and recording IC signals from cortical structures that have been subjected to ischemic stroke. **Materials and Methods:** Four male, Sprague-Dawley rats (age: 12 weeks, weight: 372-402 g) were divided into two groups of two animals; *Intervention* (IC electrical stimulation during the initial 4 hours after stroke) and *Control* (no IC stimulation). Rats were instrumented with a cuff electrode around the sciatic nerve of the right hindlimb and a 28 channel IC electrode array was inserted into the left sensory cortical hindlimb region. Cortical responses were evoked via the cuff electrode and recorded via the IC electrode array. Ischemic stroke was induced in the center of the recording site by photothrombosis. IC stimulation was applied as biphasic constant-current pulses (frequency: 2 Hz, pulse duration: 200 μs, amplitude: 50 μA). Two recordings were conducted prior to stroke and 15 recordings after stroke onset (separated by 30 min). Each recording included evoked cortical responses from 240 sciatic nerve stimulations. The cortical responses (spikes/s) within the initial 150 ms after cuff stimulation were quantified for each IC electrode. The evoked responses were divided into two groups according to the distance from the center of the stroke; *Proximate* (distance: 2-3 mm) and *Remote* (distance: 3-4 mm). *Proximate* and *Remote* responses were averaged across the two groups of rats. **Results:** As expected, the evoked cortical response increased after ischemic stroke. The *Proximate* responses were similar for the two groups of rats (*Control* vs *Intervention*). However, for the *Intervention* group the *Remote* responses were less affected than for the *Control* group initially after ischemic stroke (time: 30-240 min after stroke, cortical evoked response for *Intervention*: 54.76-85.64 % of *Control* level). **Conclusion:** A rat model of ischemic stroke applying low-frequency IC stimulation was established. Data showed that low-

frequency IC electrical stimulation may be beneficial in salvaging cortical tissue after stroke, by reducing neuronal hyperexcitability.

Disclosures: **R. Nielsen:** None. **W. Jensen:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

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Topic: C.08. Ischemia

Support: CIHR Grant

Title: Reorganization of cerebral activity patterns during ambulation following cerebral infarction assessed with 18F-FDG PET imaging

Authors: ***J.-P. SOUCY**[1], C. PAQUETTE[2,3];
[1]Montreal Neurolog. Inst., Montreal, QC, Canada; [2]Dept. of Kinesiology & Physical Educ., McGill Univ., Montreal, QC, Canada; [3]Ctr. for Interdisciplinary Res. in Rehabil. of Greater Montreal, Montreal, QC, Canada

Abstract: A frequent and morbid consequence of stroke is alterations in gait. This results in a higher incidence of falls in stroke victims than in the general population, especially when turning or performing weight transfers, and is a major source of injuries in a generally older and frailer population. However, brain imaging during prolonged, large-scale movements in humans is technically difficult, as it cannot be performed inside a scanner. There is therefore limited information on cerebral activity patterns during ambulation both in normal subjects and in stroke patients. Here, we have assessed whether 18F-FDG PET imaging was capable of showing changes in cerebral activity linked to different ambulatory conditions in post-stroke patients as compared to normal controls. Four post-stroke subjects (average time post-stroke: 25,8 months; all with lower limb scores < 7 on the Chedoke-McMaster Scale) and four matched controls performed 2 different locomotion tasks on different days. One involved long segments (28 m) of walking in a straight line with 180° turns in-between segments, and the other walking along an irregular path with multiple turns around circulation cones. Subjects received an IV injection of 18F-FDG (185 MBq on average) at initiation of ambulation, and then walked for 40 minutes (allowing full, progressive uptake of the tracer). Within 10 minutes of task completion, they were imaged (20 minute emission scan followed by a 10 minutes transmission scan) on a Siemens

HR+ PET system. Straight-walking brain activity was subtracted from that in the multiple-turns task to generate Z-score maps. Patterns of activation were much more asymmetrical in stroke subjects than in controls in the superior parietal lobules and sensorimotor cortices, with more activity changes between the 2 conditions on the side of the stroke in those with a better functional outcome and smaller infarcts, and contralateral to the lesion in those with more functional limitations and larger infarcts. Also, whereas controls showed increased activity in the vermis during the turning task, stroke subjects showed increased activity in cerebellar hemispheres. It is therefore possible to study cerebral activation during sustained motor sequences involving large amplitude movements using 18F-FDG PET imaging, and to use this approach to evaluate normal and impaired gait. Our results show differences in the way stroke patients handle complex ambulatory patterns as compared to controls. Such information might be helpful in designing subject-specific rehabilitation programs for this category of patients.

Disclosures: J. Soucy: None. **C. Paquette:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.05/P1

Topic: C.08. Ischemia

Support: NIH/NCATS KL2 TR000102

Doris Duke Charitable Foundation 2012062

Vernon Family Trust

Title: Secondary atrophy of spared left hemisphere structures in chronic post-stroke aphasia and its relationship with language deficits

Authors: *S. XING[1,3], E. H. LACEY[1,4], X. JIANG[2], L. M. SKIPPER[1], M. E. FAMA[1], P. E. TURKELTAUB[1,4];
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Abstract: After a stroke, secondary anatomical and functional changes occur in regions previously connected to the damaged areas. Although language deficits following stroke clearly

relate to brain tissue damaged directly by the stroke, it remains unclear what distant secondary effects of the damage occur and whether these relate to deficits or may demonstrate the networks damaged by the stroke. Here, we assessed whether grey matter (GM) in structures not directly impacted by the stroke changes in chronic post-stroke aphasia, and whether GM volume in spared cortical or subcortical structures relates to language outcomes. A sample of 19 patients (age: 58.6±10.2) with chronic post-stroke aphasia and 23 age-matched healthy controls (age: 61.5±8.7) participated in the study. All patients were administered a battery of language and cognitive tests and a high-resolution 3D T1-weighted structure image was collected from all patients and controls. Voxel-based morphometry (VBM) was used to quantify differences in GM volume between patients and controls. Voxel-based Lesion-Symptom Mapping (VLSM) was performed to examine relationships between lesion distribution and GM volume in spared areas among patients. Relationships between GM changes in volumes of interest (VOIs) and language performance were examined, accounting for lesion volume and lesion distribution. The VBM analysis showed that GM volumes significantly decreased in several left hemisphere regions outside the stroke lesions (FWE corrected $P<0.05$). Increased GM volumes were not observed in either hemisphere. GM volumes of VOIs significantly decreased in left inferior gyrus-pars orbitalis (IFG_orb), caudate head and thalamus compared to that in contralateral sides and controls ($p<0.01$). Lesions within left putamen and sub-frontal tissues correlated with the caudate head atrophy, and lesions in neighboring gray and white matter tissue correlated with IFG_orb atrophy ($p<0.05$, FDR correction). In the patients, residual GM volumes in the left IFG_orb and caudate head were positively correlated with spontaneous speech, generative naming and speech fluency measures, controlling for lesion size ($p<0.05$). However, when lesion distribution was included in the model (by factoring out the most significant voxel in a VLSM analysis), no relationship between atrophy and behavior remained. The current findings show that atrophy of spared GM areas in the left hemisphere results from lesions that damage connections to these areas. These lesions also cause impairment of certain language skills, suggesting that these behavioral deficits are in part caused by disconnection of spared gray matter structures.

Disclosures: **S. Xing:** None. **E.H. Lacey:** None. **X. Jiang:** None. **L.M. Skipper:** None. **M.E. Fama:** None. **P.E. Turkeltaub:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.06/P2

Topic: C.08. Ischemia

Title: Circulating immune modulators correlate with Vitamin D concentrations after HI injury in the neonatal rat

Authors: D. LOWE[1], L. ROLLINGS[2], J. FRASER[3], J. BENTZLEY[1], X. NIE[1], B. HOLLIS[1], *I. SINGH[1], D. JENKINS[1];
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Abstract: Background: Vitamin D, a neuro-immunomodulator, is depleted during inflammatory processes and is essential for normal brain development, regulating neurotrophic factors and supporting neurogenesis, neuronal plasticity, and sensorimotor and memory functions. We have previously shown N-acetylcysteine (NAC) combined with $1,25(OH)_2$Vitamin D, improves behavioral testing and infarct volume in a neonatal HI rat model. To understand mechanisms of VitD neuroprotection further, we investigated the relationship between VitD and circulating immune modulators during the second week after HI. Leptin, a T cell regulator and AMPK inhibitor important to cell survival, has been shown to be decreased in acute stroke models, and to induce neurogenesis and angiogenesis when given as a neuroprotective agent. **Objective:** We investigated if combining Hypothermia (HYPO) with NAC or NAC and VitD would alter circulating chemokines and mediators of immune response with 11 days treatment. We hypothesized that NAC and VitD treatment would change immunoreactivity during a crucial period for transition to recovery or continued inflammation and apoptosis. **Design/Methods:** Using a standard HI model in PND 7 rats, we randomized 20 rats each group to receive saline (sham, HYPO), Hypothermia+NAC (HNAC), or HNAC+VitD ip daily for seven days followed by PO administration for four days. Circulating 25-OHVitD, leptin, and chemokines were measured in serum at sacrifice 11 days post HI. **Results:** Mean serum leptin levels were significantly lower in severe HYPO animals compared to sham ($p=0.018$) but increased with HNAC + VitD treatment ($p=0.040$). In animals with severe HI injury regardless of treatment, serum leptin concentrations were higher in animals with higher 25-OHVitD levels ($\rho=0.516$, $p=0.028$). 25-OH VitD levels negatively correlated with MCP-1 ($\rho=-0.514$, $p=0.0005$), IL-18 ($\rho=-0.412$, $p=0.005$), and RANTES ($\rho=-0.322$, $p=0.037$) in all hypothermic HI animals, regardless of treatment or severity. However, VitD treatment did not result in lower MCP-1, IL-18 or RANTES, suggesting VitD does not directly decrease these mediators of chronic inflammation. **Conclusions:** Our data suggest that HNAC+VitD treatment increases serum leptin concentrations over hypothermia alone, and may contribute to improved outcomes.

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Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

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Program#/Poster#: 702.07/P3

Topic: C.08. Ischemia

Support: AHA EIA 0940065N

NINDS RO1 NS071050

Title: An aberrant increase of brain-state alternation after experimental stroke

Authors: *J. W. HE[1,2], Y. NISHIJIMA[1,2], Y. AKAMATSU[1,2], J. LIU[1,2];
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Abstract: Patterns of brain oscillation are not only good indicators of cognitive function but also predictors of functional outcome in patients with ischemic stroke. However, little is known about the role of brain oscillation in stroke-induced cognitive dysfunction in the hippocampus, a remote brain region that suffers from functional impairment, despite no structural damage, following distal occlusion of the middle cerebral artery (dMCAO). Our previous studies have shown that rats subjected to dMCAO, consisting of a permanent occlusion of unilateral MCA and temporary occlusion of the bilateral common carotid artery (CCAO) for 60 min, exhibited impaired spatial learning and memory that coincided with a region-specific reduction in neuronal activation in the hippocampus. The current study aimed to determine the temporal changes of brain oscillation following stroke. Extracellular intracortical and hippocampal electrical activity was recorded bilaterally in Sprague Dawley rats under urethane anesthesia with 16- and 32-channel Neuronexus probes. Compared to sham operation, dMCAO increased the alternation frequency between the theta and slow-wave states (high delta power and high occurrence of ripples) during occlusion and the subsequent reperfusion of the CCA. Such an increase in the brain-state alternation was also found in rats with CCAO alone, but only during occlusion and to a lesser extent. As the brain-state reflects network activity across the brain upon which hippocampal function critically depends, the aberrant increase in the brain-state alternation in the hippocampus may indicate a functional disruption between the hippocampus and its afferent connections. Our results suggest that the pathological increase in the brain-state alternation might be a novel biomarker for stroke-induced connectivity change and functional impairment.

Disclosures: J.W. He: None. **Y. Nishijima:** None. **Y. Akamatsu:** None. **J. Liu:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

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Program#/Poster#: 702.08/P4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant RO1 HL091541-18 WAB

Title: Dendrimer nanotherapies for the treatment of brain injury following hypothermic circulatory arrest in a large animal model

Authors: *M. K. MISHRA[1], C. A. BEATY[2], W. G. LESNIAK[1], S. P. KAMBHAMPATI[1], F. ZHANG[1], M. A. WILSON[3], M. E. BLUE[3], S. KANNAN[4], M. V. JOHNSTON[5], W. A. BAUMGARTNER[2], K. M. RANGARAMANUJAM[1];
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Abstract: Hypothermic circulatory arrest (HCA) can lead to neurological complications including stroke, seizures, neurocognitive dysfunction, and delayed neuropsychomotor development in many patients. Neuroinflammation and excitotoxicity have been identified as key factors leading to the brain injury after HCA. The treatment has remained a challenge, with no suitable therapeutic options. Valproic acid (VPA) has been explored in this model, and offers some neuroprotection against excitotoxicity with a large dose, but leads to systemic side effects. Similarly *N*-Acetylcysteine (NAC) is known to be effective in reducing neuroinflammation, but large doses are required due to poor blood-brain barrier (BBB) penetration and insufficient brain localization. To overcome these challenges, we are exploring systemic, combination therapies targeted to activated microglia and injured neurons, using dendrimers. We use a well-established, clinically relevant canine model.[1] Using fluorescently labeled, hydroxyl-terminated polyamidoamine (PAMAM) dendrimers, we showed that systemically administered dendrimers can cross the impaired blood-brain-barrier, and target activated microglia and injured neurons, with minimal uptake in the healthy cells in the brain.[1] Based on above mentioned findings, we developed glutathione-sensitive dendrimer-NAC (D-NAC) and dendrimer-VPA (D-VPA) conjugates in multi-gram quantities for fast intracellular release. In preliminary efficacy studies, combination therapy with D-NAC and D-VPA produced improvement in 24-hour neurological deficit score comparable to combination therapy with VPA and NAC at 10-30 fold higher doses, while significantly reducing the adverse side effects. This study highlights the potential of dendrimer-drug therapies of two clinically approved drugs in HCA-indued large animal model.

These are the first dendrimer nanotherapy studies in a large animal brain injury model.
References 1. Dendrimer brain uptake and targeted therapy for brain injury in a large animal model of hypothermic circulatory arrest.Mishra MK, Beaty CA, Lesniak WG, Kambhampati SP, Zhang F, Wilson MA, Blue ME, Troncoso JC, Kannan S, Johnston MV, Baumgartner WA, Kannan RM. **ACS Nano**. 2014 Mar 25;8(3):2134-47.

Disclosures: M.K. Mishra: None. **C.A. Beaty:** None. **W.G. Lesniak:** None. **S.P. Kambhampati:** None. **F. Zhang:** None. **M.A. Wilson:** None. **M.E. Blue:** None. **S. Kannan:** None. **M.V. Johnston:** None. **W.A. Baumgartner:** None. **K.M. Rangaramanujam:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.09/P5

Topic: C.08. Ischemia

Support: CIHR Grant MOP-12675

CIHR Grant MOP-111009

Title: Optogenetic mapping after focal somatosensory cortex stroke reveals network-wide plasticity and regionally-scaled recovery of functional connectivity

Authors: *D. H. LIM[1], J. LEDUE[1], M. H. MOHAJERANI[2], T. H. MURPHY[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: We used arbitrary point channelrhodopsin-2 (ChR2) stimulation and wide-scale voltage sensitive dye (VSD) imaging to map altered cortical connectivity at 1 and 8 weeks post-stroke within the affected and contralesional hemisphere. Sensory- and ChR2-evoked maps were depressed within the peri-infarct cortex, although delayed responses were only observed for sensory stimulation. At 1 week after stroke we observed a depression of responsiveness that extended to the uninjured hemisphere; at 8 weeks significant recovery was observed. Network analysis revealed a symmetrical sham network of mirrored hemispheric modules, within which were sensorimotor and association modules. This symmetry and modularity was disrupted after stroke, however, when we scaled the ChR2-evoked VSD responses from the stroke groups to match the sham group mean we found the distribution of responses were indistinguishable. This

suggests network-wide scaling after stroke with a decreased responsiveness relative to sham levels. Closer inspection of the peri-infarct at 1 and 8 weeks after stroke using a fine grid of stimulation points indicated a dissimilar distribution of responses that persisted even when scaling to match the sham mean was applied. Our findings are consistent with a model where focal stroke has relatively wide-reaching effects on most points within the cortical network. During recovery most cortical areas undergo an equally-scaled homeostatic change, resulting in a relative distribution of responses that is similar to the sham network, albeit depressed. In contrast, recovery in the peri-infarct is heterogeneous and these cortical points do not follow a strict scaling factor expected for the entire network.

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Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.10/P6

Topic: C.08. Ischemia

Support: Air Force Medical Support Agency G1709Y14

Title: High altitude exposure and psychological stressors in post-traumatic stress disorders

Authors: *N. P. CRAMER[1], X. XU[1], A. BIERMAN[2], C. TANKERSLEY[2], Z. GALDZICKI[1]; [1]Anatomy, Physiol. and Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]Bloomberg Sch. of Publ. Hlth., Johns Hopkins Univ., Baltimore, MD

Abstract: Harsh environmental conditions, such as those experienced at high altitudes (HA), may interact with psychological stressors to potentiate the mechanisms responsible for post-traumatic stress disorders. Using a mouse model of HA exposure we are investigating the potential epigenetic links between environmental and psychological stress. Mice with two different genetic backgrounds, BALBc and C57Bl6 (B6), were exposed to simulated HA (5000 m above sea level) in a custom-built hypobaric chamber for up to 3 months. Environmental parameters, including O2, CO2, temperature and humidity, were monitored during exposures. Physiological signs of stress were examined by histology and whole-body plethysmography. Behavioral signs of stress were examined using the rotarod, treadmill, and acoustic startle response. Transcriptome changes were examined using RNAseq of tissue isolated from the

hippocampi and amygdalae. Upon HA exposure, both strains of mice become sessile and lose weight. This weight loss fails to recover to that of control mice. On the rotarod, HA exposed mice outperform controls and this effect is more pronounced in BALBc mice. Whole body plethysmography suggests that initial HA induced changes in the neural control of breathing normalizes over time while other maladaptive changes in lung physiology occur. However, gross pathological examination of H&E stained lung tissue did not uncover any abnormalities in HA mice suggesting functional changes result from more subtle cellular or structural changes. This model provides an excellent platform for investigating the degree to which environmental stressors facilitate the onset of PTSD-like symptoms and the epigenetic consequences of these interactions.

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Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

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Program#/Poster#: 702.11/P7

Topic: C.08. Ischemia

Support: PHRC ISIS 07PHR04. RCB 2007-A00853-53

PHRC HERMES 10PHR26-04

Title: Ipsilesional corticospinal tract damage assessed with diffusion imaging predicts sensorimotor recovery

Authors: *A. JAILLARD[1,3], F. RENARD[6], O. DETANTE[2,4], T. ZEFFIRO[7], F. ISIS HERMES GROUP[5];
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Abstract: Purpose: Accurately determining prognosis concerning the final level of recovery after ischemic stroke based on standard clinical data available at stroke onset is difficult. To determine the value of diffusion tensor imaging (DTI) in acquiring structural biomarkers

predicting motor recovery after stroke, we collected subacute DTI measures of corticospinal tract (CST) integrity and tested whether those measures predicted clinical motor performance six months later. Methods: Twenty-eight patients (mean age 51 ± 9 years) with stroke involving sensorimotor cortex and varying degrees of associated sensorimotor impairment underwent DTI and clinical sensorimotor performance assessments one and six months after onset. Comparisons were made to 23 healthy controls (mean age 41 ± 15 years). Clinical tests including NIHSS, Barthel, and Fugl-Meyer were administered along with DTI (60 non-collinear diffusion gradient directions, voxel size=1.6x1.6x2.0 mm3 and b-value = 1000 s/mm2) and T1-weighted structural volumes (1 mm3 voxels). Preprocessing included gradient scheme, eddy-current and head motion correction. FA parametric images were estimated and non-linearly registered to the MNI-152 standard space. To quantify CST damage, VOIs were manually delineated in both ipsilesional (IL) and contralesional (CL) CSTs, remote from the site of direct damage, at the midbrain level. CST lesion volume across all levels was assessed using the T1-weighted volumes and mean FA was computed from the DTI data. IL and CL CST measures were assessed using multivariate analysis. Linear regression was used to explore the predictive value of the DTI measures on clinical recovery scores. Results: Patients exhibited lower FA in the IL compared to the CL CST ($p< 0.001$) or the controls' CST ($p< 0.001$). In patients, IL CST FA measured one month after injury successfully predicted the NIHSS ($p=0.008$), motor NIHSS ($p=0.001$) and Barthel scale scores ($p<0.001$) measured at six months. Conclusions: As reported previously, we observed significant Wallerian degeneration after unilateral stroke in the IL compared to the CL CST and control CST. IL CST reductions predicted clinical status at six months. As the mean FA was measured within the undamaged part of the CST, its reduction probably reflects Wallerian degeneration. FA measures derived from subacute DTI data may provide sensitive and clinically practical biomarkers to predict the eventual level of motor recovery following stroke.

Disclosures: A. Jaillard: None. **F. Renard:** None. **O. Detante:** None. **T. Zeffiro:** None. **F. ISIS HERMES Group:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.12/P8

Topic: C.08. Ischemia

Support: HL107640

Title: Exosomes derived from curcumin-primed stem cells as novel therapeutic vehicles in diabetic stroke

Authors: *A. KALANI[1], P. K. KAMAT[2], S. C. TYAGI[2], N. TYAGI[2];
[1]Physiol. and Biophysics, [2]Univ. of Louisville, Louisville, KY

Abstract: Stroke during diabetes is severe in terms of extensive neuro-glio-vascular dysfunction. The therapy available (tissue plasminogen activator) till date, is not effective in diabetic stroke and leads to excessive vasodilation and hemorrhagic transformations. Curcumin possesses remarkable medicinal properties but its bioavailability is a major concern. Exosomes (nano-vesicles; <100nm) derived from stem cells have paracrine effects, neo-vascularization properties and excellent ability to cross blood-brain barrier. Therefore, we tested the hypothesis whether the combination of exosomes and curcumin (curcumin-primed exosomes) mitigate diabetic stroke severity. Ischemia reperfusion (*I/R*) injury was induced in 8 weeks old male wild type (C57Bl/6J; *I/R*) and diabetic heterozygous *Ins2*^{Akita} (C57BL/6-*Ins2*^{Akita}/J; *I/R*^{Akita}) mice groups. Exosomes were isolated from conditioned culture media of curcumin-primed mouse embryonic stem cells (CUR-EXO) and used for the treatment through intranasal route. Infarct area was measured by triphenyltetrazolium chloride (TTC). Brain cryo-sections were analyzed for glia coupling (Cx43, GFAP, CD11B) in ipsilateral cortical vessels. Insulin sensitivity in cortical neurons was checked by Western blot while neuronal loss, neurodegeneration was analyzed with NeuN, fluoro-jade C staining. White matter damage was examined with luxol fast blue and bielschowsky silver staining. Dual-tracer probing method was used to analyze cerebrovascular permeability and passive avoidance test was used to measure memory function. Infarct volume was greater in *I/R*^{Akita} mice following MCAO as compared to *I/R* mice. Extensively reduced glial markers in cortical vessels, neurodegeneration and marked reduction in neuronal insulin sensitivity were observed in *I/R*^{Akita} as compared to *I/R* mice. White matter, axon-glia damage and permeability were exacerbated in *I/R*^{Akita} as compared to *I/R*. Severity of memory deficit was more in *I/R*^{Akita} as compared to *I/R*. Interestingly, treatment with CUR-EXO ameliorated neuro-glio-vascular coupling and permeability in diabetic mice. These results suggest that curcumin-primed stem cells exosomes improve neuronal-glial-vascular and cognitive functions and represents a novel treatment for stroke during diabetes.

Disclosures: **A. Kalani:** None. **P.K. Kamat:** None. **S.C. Tyagi:** None. **N. Tyagi:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.13/P9

Topic: C.08. Ischemia

Support: CIHR

Canadian Partnership for Stroke Recovery

Title: Development of a mouse model of diffuse microinfarction and automated assessment tools for monitoring recovery

Authors: *G. SILASI[1], F. BOLANOS[1], J. SHE[1], J. BOYD[1], J. LEDUE[1], M. VANNI[1], S. H. SCOTT[2], T. H. MURPHY[1];
[1]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [2]Dept. of Biomed. and Mol. Sci., Queens Univ., Kingston, ON, Canada

Abstract: The need for reliable animal models of vascular dementia is great, as the vascular and neuronal changes that produce this condition in humans are difficult to study in patient populations. Our approach has been to develop a mouse model of diffuse microinfarction that is compatible with recently developed optogenetic tools and head-fixed behavioural tasks in mice. To induce diffuse microinfarction, 20um fluorescent microspheres are injected unilaterally into the common carotid artery of anesthetized mice. We found that injecting ~2,000 beads results in 629 (+/-225) beads lodging in the injected hemisphere, with approximately half (45%) lodged in cortical vessels. Preliminary analyses show that microinfarcts in the CA fields of the hippocampus and thalamic nuclei often produce cell death and loss of dendritic structure, while no such obvious changes are observed in the neocortex. We are currently performing detailed analyses of spine density and dendritic morphology. Mice are also trained to pull an actuated lever (KINPAW) to a target position to receive a water reward. After training, mechanical loads are randomly applied and the mice must correct for these loads and return the lever to the original target position. Detecting and correcting for this perturbation requires active cortical processing and sensorimotor integration. During home-cage training mice perform 500-1000 trials in 24 hrs, and can learn to hold the lever in the target position for over 3 seconds. We are currently assessing the ability of the mice to correct for perturbations and will characterize the performance of head-fixed mice with microinfarcts. In contrast to most cognitive tasks where behavioural impairments are only observed after bilateral injury, we feel that the ability to assess motor performance on a large number of trials will be a valuable asset for identifying behavioural deficits in our unilateral model of diffuse microinfarction.

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Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.14/P10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sox11 is a novel inhibitor of caspase-6 activity

Authors: *E. A. WALDRON[1], J. HOEURAUF[1], N. ARBEZ[2], S. ZHU[2], F. NUCIFORA[2], K. KULSCAR[3], C. A. ROSS[2];
[1]Psychiatry, Div. of Neurobio., [2]Johns Hopkins Univ., Baltimore, MD; [3]Johns Hopkins Univ., Baltimore, MA

Abstract: The apoptotic cascade is an orchestrated event, whose final stages are mediated by effector caspases. Regulatory binding proteins have been identified for caspases such as caspase-3, -7, -8, and -9. Many of these proteins belong to the inhibitor of apoptosis (IAP) family. By contrast, caspase-6 is not believed to be influenced by IAPs, and little is known about its regulation. We therefore performed a yeast-two-hybrid screen using a constitutively inactive form of caspase-6 for bait in order to identify potential novel regulators of caspase-6 activity. Sox11 was identified as a potential caspase-6 interacting protein. Sox11 was capable of dramatically inhibiting caspase-6 activity. Sox11 appears to block caspase-6 dimerization and thereafter caspase-6 self-cleavage. Several regions, including amino acids 117-214 and 362-395 within sox11 as well as a nuclear localization signal (NLS) all contributed to the inhibition of caspase-6 activity. Furthermore, sox11 was also capable of inhibiting other effector caspases but not initiator caspases -8 and -9. The ability of sox11 to inhibit effector caspases was also reflected in its capacity to reduce cell death following toxic insult. Interestingly other sox proteins also had the ability to inhibit caspase-6 but to a lesser extent than caspase-6. Sox11's ability to inhibit effector caspases might be relevant to cancer, since carcinogenesis involves in part an escape from normal apoptosis. Furthermore activation of effector caspases is also a key event mediating neuronal death in neurodegenerative disease. To date, no protein inhibitor has been described for caspase-6. Identification of inhibitory interactions, in combination with structural information, could potentially provide clues to the design of new classes of small molecule inhibitors which could be used to treat disease.

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Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA 3R01AA020103

NIGMS COBRE P30 GM103328

Intramural Research Support grant from the University of Mississippi Medical Center

Title: Sp1 mediates ethanol-induced KLF11 gene expression

Authors: *S. L. SWILLEY[1], S. JOHNSON[1], J. DUNCAN[1], P. R. ALBERT[4], J. LOU[5], D. B. SITTMAN[2], X.-M. OU[1], C. A. STOCKMEIER[1], J. WANG[3];
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Abstract: Alcohol abuse leads to brain disorders that are associated with neurodegeneration and brain cell death. Our recent work suggests that increased expression and activity of monoamine oxidase B (MAO B) contributes to ethanol -related brain neurodegeneration. Increased MAO B activation produces excessive reactive oxygen species, a byproduct of its enzymatic degradation of neurotransmitters, and causes cell death. However, the molecular mechanisms associated with ethanol-induced MAO B activation are not fully known. The transcription factor Kruppel-like factor 11 (KLF11, also known as TIEG2) regulates MAO B expression by binding to Sp1 binding sites in the MAO B promoter and plays a role in cell apoptosis. Therefore, we hypothesis that ethanol- induced KLF11 expression is involved in the ethanol- mediated MAO B- cells death pathway. In this work, we seek to investigate the molecular mechanisms involved in increased KLF11 expression following ethanol exposure by examining the role of Sp1, a transcriptional regulator of MAO B and an ethanol responsive gene, in basal and ethanol-induced KLF11 promoter activation in SH-SY5Y cells. SH-SY5Y cells were transfected with the full length KLF11 (1.5 kb) or serial deletions of the KLF11 promoters fused to the pGL4 luciferase reporter gene vector (Promega). Following transfection, the cells were treated either with ethanol (75 mM, 24 hours) or control, and luciferase activities were determined. In parallel, the Sp1 expression vector or pCMV empty vector were co-transfected with the 1.5 kb KLF11 promoter or serial deletions of the KLF11-luciferase reporter vector and treated with the ethanol or control and Sp1 binding to the SP1 binding domain in KLF11 promoter were assessed. Our results demonstrate that overexpression of Sp1 leads to an ethanol-induced increase in KLF11

expression. KLF11 promoter luciferase activity assays revealed that the distal Sp1 binding elements in the KLF11 promoter are necessary for basal and ethanol-induced activation of KLF11. Sp1 binding to the distal Sp1 binding sites increased ethanol-induced KLF11 promoter activation. These findings establish a role for Sp1 in ethanol-induced KLF11-MAO-B mediated cell death and provide further insight into the molecular mechanisms responsible for the neurotoxicity associated with alcohol abuse.

Disclosures: S.L. Swilley: None. **S. Johnson:** None. **J. Duncan:** None. **P.R. Albert:** None. **J. Lou:** None. **D.B. Sittman:** None. **X. Ou:** None. **C.A. Stockmeier:** None. **J. Wang:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.16/P12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Regulation of System xc- by TGF-β1

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Abstract: System xc-, the cystine/glutamate exchanger located on the cell membrane, mediates the transport of one cystine molecule into the cell in exchange for the release of one glutamate molecule into the extra extrasynaptic space. Through providing cystine to the cell system xc- regulates the levels of cellular glutathione (GSH), the main endogenous intracellular antioxidant, and in this way may protect cells against oxidative stress. However, by releasing glutamate, it can increase extracellular glutamate levels and potentially cause excitotoxicity. Due to this dual nature, system xc- likely plays an important role in regulating neuronal survival and death. There is also evidence that both excitotoxicity and oxidative stress play key roles in neurodegenerative diseases, like amyotrophic lateral sclerosis (ALS) and Alzheimer's disease; thus, system xc- may play a key role in the pathogenesis of these diseases. In order to better understand the role system xc- plays in neuronal survival and death we must first understand how it is regulated. We have found that transforming growth factor β1 (TGF-β1) increases cystine uptake through system xc- in both a concentration and time-dependent manner in glial cortical cultures. TGF-β1 is involved in many cellular processes, including cellular proliferation, differentiation, and apoptosis; studies have shown TGF-β1 signaling dysfunction in both ALS and Alzheimer's disease. We are currently assessing the mechanism through which TGF-β1 increases cystine uptake through

system xc-, and have thus far determined that the upregulation of system xc- by TGF-β1 is ERK-dependent. We have also found that neuronal conditioned media (NCM) causes an upregulation of system xc-. In these experiments media is taken from neuron-enriched cortical cultures and placed on glial cortical cultures. After 24 hours there is an increase in system xc- mediated cystine uptake into the glial cells. These experiments suggest that neurons release a substance that upregulates system xc- on glial cells. We are currently working on determining what this substance is and the mechanism through which it is upregulating system xc-. Specifically, TGFβ1, fibroblast growth factor-2 (FGF-2), and pituitary adenylate cyclase-activating polypeptide (PACAP), substances known to be released by neurons, and that we have previously shown to upregulate system xc- in this system, are being studied to determine if they are responsible for the effects induced by NCM.

Disclosures: R. Albano: None. **J. Hjelmhaug:** None. **D. Lobner:** None. **X. Liu:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.17/Q1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Characterization of the kallikrein-kinin system post chemical brain injury: An *in vitro* biochemical approach

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Abstract: Background: Traumatic Brain Injury (TBI) is the result of a mechanical impact on the brain that can provoke mild to moderate to severe symptoms. It is acknowledged that TBI leads to apoptotic and necrotic cell death; however, the exact mechanism by which brain trauma leads to neuronal injury is not fully elucidated yet. Noteworthy, the Kallikrein Kinin System (KKS) represents the first inflammatory pathway activated following tissue injury. Thus, the KKS's contributing role represents a good target to assess in the area of TBI. Aims: The aim of this study is to investigate the expression and the role of the KKS's main players: Bradykinin and its receptors, in mediating neuronal injury. This will be evaluated under chemical (*in vitro*) neurotoxicity paradigms. The neuronal cell line PC12 will be treated with the apoptotic drug Staurosporine (STS) to simulate the cell activity after TBI to a certain degree. Methods: The

effect of STS on the viability and proliferation of pre-treated PC12 cells was investigated using MTT, ROS and LDH assays. DAPI nuclear staining was performed to assess for apoptotic bodies. MitoPT JC-1 and Annexin-V staining were presented as a proof of apoptosis. Intracellular calcium release was evaluated by Fluo 4-AM staining. Immunofluorescence (IF) was also performed on KKS markers, notably bradykinin 1 and 2 receptors (B1R and B2R). Western blotting (WB) was performed on both apoptotic and KKS markers. Real-time PCR, IF and WB were conducted to study the effect of STS on the transcriptional, translational and cellular localization of KKS markers. Finally, proteomics was used to find relevant proteins associated to STS and KKS in PC12 cells. Results: STS inhibited the proliferation of pre-treated PC12 cells in a time-dependent manner. DAPI, WB and IF confirmed that apoptosis is the mechanism that STS is inducing and that it is mainly the B2R that is being altered after exposure to STS chemical neurotoxicity. Interestingly, inhibition of the B2R was shown to prevent calcium release following STS treatment and was showing the highest transcriptional level 3h and 12h post chemical injury. Proteomics results confirmed the emergence of a "survival" capacity developed by the cell when treated with B2R inhibitor and STS. Conclusion: Our data suggests that the B2R is a main player in the inflammatory pathway following STS-mediated apoptosis in PC12 cells and its inhibition represents a potential therapeutic tool.

Disclosures: F.H. Kobeissy: None. **A.M. Nokkari:** None. **T. Mouhieddine:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

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Program#/Poster#: 702.18/Q2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DGAPA IN219511 S. R-A

Title: The effect of oxidative stress on CB1 and CB2 receptors and their relationship with the PPARγ in substantia nigra of rats exposed to ozone

Authors: *G. E. GOROSTIETA SALAS, R. VELÁZQUEZ PÉREZ, S. RIVAS ARANCIBIA; Deprtamento de Fisiología, UNAM, Mexico City, Mexico

Abstract: The loss of oxide-reduction balance in the organism that involves the increase of the pro-oxidant molecules and an impaired on the antioxidant systems produces a state of oxidative stress. The exposition to low doses of ozone (O_3) produces such a state, and this condition alters

inflammatory response, leading to tissue damage. Previous experiments in our laboratory show that after 30 days of exposition to ozone, a progressive and irreversible neurodegenerative condition was observed in the central nervous system. On the other hand, the endocannabinoid system is an important modulator of the immune system and several studies show that this system is modified under oxidative stress conditions, suggesting a regulating role to the inflammatory process, probably through the activation of the nuclear PPARγ receptors. The objective of this work was to evaluate the effect of oxidative stress in the substantia nigra of rats exposed to O_3. Thirty-six male rats were individually housed in acrylic boxes with free access to food and water. The animals were divided randomly into 6 groups; 1) control, 2) O_3 7 days, 3) O_3 15 days, 4) O_3 30 days, 5) O_3 60 days, 6) O_3 90 days. The O_3 was administered at doses of 0.25 ppm 4 hours per day. The control group was exposed to air free of ozone. After the treatment, the animals were deeply anesthetized and tissue was prepared for immunohistochemical assay and western blot. The sc-25494 antibody and ab23703 were used for the inmmunodetection. The results show an increase in immunoreactivity at 30, 60 and 90 days of the CB1 and CB2, when the results were compared with the controls. In conclusion, chronic exposure to low doses of ozone modifies the expression of CB1 and CB2 receptors, suggesting a compensatory mechanism against the damage induced by ozone.

Disclosures: **G.E. Gorostieta Salas:** None. **R. Velázquez Pérez:** None. **S. Rivas Arancibia:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

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Program#/Poster#: 702.19/Q3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Multiple Sclerosis Society of Canada

Canadian Institutes of Health Research

Title: Early cell death in oligodendrocytes measured by spectral changes of the fluorescent nuclear dye acridine orange

Authors: ***J. R. PLEMEL**, M. B. KEOUGH, I. MICU, E. R. J. MANFORD, V. W. YONG, P. K. STYS;

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Abstract: Cell death is a feature of all central nervous system trauma and disease. To study cell death, it is possible to model the normal central nervous system cytoarchitecture with culturing tissue slices or short-term maintenance of *ex vivo* samples. However, the thickness of tissue slices and *ex vivo* samples preclude the use of common antibody-based approaches to measure cell death in living samples. Deep penetrating cell-impermeant nuclear dyes such as propidium iodide offer one strategy to label dead cells, but these dyes label a very late stage of apoptosis/necrosis and are thus not sensitive to detect early stages of injury. In the hopes of detecting early apoptotic events we measured the spectral changes of a nuclear dye, acridine orange, under normal and pathological conditions in cell culture. A human oligodendroglial cell line and mouse oligodendrocytes were cultured with or without apoptosis and necrosis inducing agents and stained with acridine orange. Acridine orange emits in the green spectrum when bound to double-stranded DNA and in the red spectrum when bound to RNA or single-stranded DNA. We found that cell death induces a spectral change in cells, with less nuclear red emission. Importantly, the spectral change of acridine orange preceded the entry of a cell impermeant dye, propidium iodide, in agreement with our hypothesis that acridine orange is a sensitive dye for early cellular damage. Imaging of acridine orange could provide a novel strategy to measure cell death in live samples allowing additional mechanistic interrogation of early cell injury.

Disclosures: **J.R. Plemel:** None. **M.B. Keough:** None. **I. Micu:** None. **E.R.J. Manford:** None. **V.W. Yong:** None. **P.K. Stys:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

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Program#/Poster#: 702.20/Q4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: LKR R01AT006880

Title: Maternal inflammation is associated with increased Nurr1 expression in the developing brain

Authors: *A. E. GRAF[1,2], S. W. LALLIER[1], J. P. GODBOUT[3], L. K. ROGERS[1];
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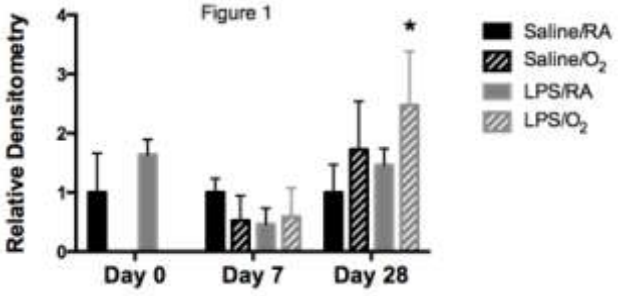
Abstract: Nearly half of babies born prematurely will experience lifelong neurodevelopmental impairment (NDI). The majority of these impairments are cognitive. Maternal inflammation and neonatal hyperoxia are key risk factors for NDI. Nuclear receptor-related 1 (NURR1, NR4A2) is a transcription factor expressed in dopaminergic and subplate neurons that may also confer neuroprotection by attenuating inflammatory cytokine release from microglia. Nonetheless, the effect of perinatal inflammation on Nurr1 expression in the developing mouse brain is unclear. The purpose of this study was to begin to test the hypothesis that Nurr1 protein expression is increased by microglia in response to perinatal inflammation. In these experiments, pregnant C3H/HeN mice were injected with 80µg/kg of lipopolysaccharide (LPS) or saline on embryonic day 16 and newborn pups exposed to room air (RA) or 85% oxygen (O_2) for fourteen days. Nurr1 protein expression was determined from whole brain homogenates at postnatal days 0, 7, and 28. Here we show that on postnatal day 0, pups from LPS-injected dams had increased Nurr1 expression. By day 7, during O_2 exposure, however, this increase was absent and instead the Saline/O_2, LPS/RA, and LPS/O_2 treated pups displayed a reduction in Nurr1 expression. After the period of RA recovery, at day 28, Nurr1 expression is significantly increased in the LPS/O_2 treatment group (Figure 1, p=0.03, n= 1 pup each from 3-5 litters/treatment group). Therefore, Nurr1 expression is increased in LPS/O_2 exposed mice in adolescence after a period of RA recovery. Additional studies are ongoing and are aimed at identifying the areas and cell types where Nurr1 expression is concentrated and the patterns of expression in these cells specifically in response to perinatal inflammation. Understanding the effects of inflammation on Nurr1 expression and subsequent NDI may provide a framework for investigating the role of Nurr1 as a potential therapeutic target in high-risk preterm neonates.



Disclosures: **A.E. Graf:** None. **J.P. Godbout:** None. **S.W. Lallier:** None. **L.K. Rogers:** None.

Poster

702. Ischemia: Pathophysiology, Biomarkers, and Treatment

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 702.21/Q5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Transcriptional analysis of apoptotic cerebellar granule neurons following rescue by gastric inhibitory polypeptide

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Abstract: Apoptosis triggered by exogenous or endogenous stimuli is a crucial phenomenon to determine the fate of neurons, both in physiological and in pathological conditions. Our previous study established that gastric inhibitory polypeptide (Gip) is a neurotrophic factor capable of preventing apoptosis of cerebellar granule neurons (CGNs), during its pre-commitment phase. In the present study, we conducted whole-genome expression profiling to obtain a comprehensive view of the transcriptional program underlying the rescue effect of Gip in CGNs. By using DNA microarray technology, we identified 65 genes, we named survival related genes, whose expression is significantly de-regulated following Gip treatment. The expression levels of six transcripts were confirmed by real-time quantitative polymerase chain reaction. The proteins encoded by the survival related genes are functionally grouped in the following categories: signal transduction, transcription, cell cycle, chromatin remodeling, cell death, antioxidant activity, ubiquitination, metabolism and cytoskeletal organization. Our data outline that Gip supports CGNs rescue via a molecular framework, orchestrated by a wide spectrum of gene actors, which propagate survival signals and support neuronal viability.

Disclosures: **B. Maino:** None. **M. Ciotti:** None. **P. Calissano:** None. **S. Cavallaro:** A. Employment/Salary (full or part-time):; Institute of Neurological Science-National Research Council, Institute of Cellular Biology and Neurobiology-National Research Council.

Poster

703. Traumatic Brain Injury: Human Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 703.01/Q6

Topic: C.10. Trauma

Title: A robust multichannel method for estimation of EEG power spectra and coherences

Authors: *T. MELMAN[1], N. SCHIFF[2], J. VICTOR[2];
[1]Weill Cornell Grad. Sch. of Med. Sci., New York, NY; [2]Weill Cornell Med. Col., New York, NY

Abstract: Among the many techniques for non-invasive measurement of brain activity, electroencephalography (EEG) occupies a unique niche: it has high temporal resolution, it is a direct assay of neuronal activity, and it is a widely-available technology that can readily be applied to severely ill patients. These features make it a good option for studying patients with neurologic disorders, including severe brain injury. However, EEG recordings often contain many kinds of artifacts, including electrical signals originating in non-neural sources from the patient and the environment. This noise often obscures the underlying signal, posing a challenge to quantitative analysis. As a consequence, EEG signal processing requires hand-cleaning data to remove segments with artifact prior to further analysis. The end result is reasonably accurate, but the process is tedious, especially when artifact is frequent. Focusing on estimation of the power spectrum and coherence, we describe and demonstrate an alternative to this data pipeline that reduces the impact of artifacts by using robust statistical methods, which often provide more stable estimates of a distribution's center and shape when the distribution contains outliers. In the standard approach to spectral estimation, the final step takes the average of squares of the Fourier coefficients. It has been noted (Wong et al., 2011 IEEE EMBS) that replacing the mean of these squared values by a robust estimator, the median, yields cleaner estimates of the spectrum and coherence. This generic application of robust methods is proof of principle, but ignores two important aspects of the spectral estimation problem. (i) It is intrinsically multivariate - geometrically, the problem reduces to estimating the shape of a multidimensional cloud of Fourier components, with one complex dimension for each channel. (ii) Because the reference time for the analysis is chosen arbitrarily, the distribution of Fourier coefficients in each complex plane is circularly-symmetric, with further symmetry constraints on their covariances. Here we show how these characteristics can be exploited via robust statistics tailored to the problem of spectral estimation. We begin with a generic robust estimator of shape, for example the Minimum Covariance Determinant (MCD, Rousseeuw 1984), which exploits the multivariate nature of the problem. We then modify the shape estimator to utilize the symmetry considerations associated with time series analysis. Simulations show that this is an effective approach for estimating the power spectrum and covariance of the underlying EEG in the presence of substantial noise.

Disclosures: **T. Melman:** None. **N. Schiff:** None. **J. Victor:** None.

Poster

703. Traumatic Brain Injury: Human Studies

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Topic: C.10. Trauma

Support: NIH Grant 5RO1-HD51912

James S. McDonnell Foundation

Title: qEEG evidence for preservation of the auditory working memory buffer in severely brain injured subjects

Authors: H. M. MARKELL[1,2], L. F. MENDELS[2], *M. M. CONTE[3], N. D. SCHIFF[2]; [1]Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA; [2]Brain & Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [3]Brain & Mind Res. Inst., New York, NY

Abstract: Aspects of linguistic processing have been shown to localize to distinct cortical regions. Early auditory cortices (A1) respond reliably for all low-level auditory stimuli, while higher-order areas are specialized for processing of syntactic structure building into natural language comprehension. Specifically, several studies have found that presentation of an intact narrative demonstrated greater fMRI activity in left and right temporal regions, compared to a time-reversed story or an otherwise unintelligible group of sounds, (Crinion & Price, 2005). Furthermore, findings have indicated differential activity even when language is comprehensible. Comparisons of unconnected sentences to an intact narrative revealed greater activation of other higher order processing areas, including anterior temporal regions, medial prefrontal regions and the precuneus (Lerner et al., 2011; Rogalsky, et al., 2011). It has been established via EEG studies that language processing is associated with increased narrowband power in the theta frequency range (4-7 Hz) (e.g. Bastiaansen et al., 2005). Here, we use Lerner et al.'s (2011) narrative paradigm to examine processing of language using hierarchical stimuli. We focus our analysis on several EEG channels of interest in bilateral frontal and anterior temporal regions, as determined by earlier studies. The EEG was recorded in 16 healthy controls (10 M; ages 19-52) and two patient subjects (2M, ages 29, 23) who sustained severe traumatic brain injuries. One patient subject recovered reliable gestural communication; the other shows consistent communication via a novel non-gestural mode (see Mendels et al. this meeting). All subjects listened to three versions of a live-recorded story ("Pie-man"): 1) waveform-reversed in time (Bkwd) in which the words were not intelligible, 2) a sentence-scrambled version (SS), and 3) the intact story from beginning to end (Fwd). Power spectral analysis of the EEG showed

increased power in the theta frequency range in the fronto-central regions bilaterally when the Fwd and SS conditions were compared to the Bkwd condition for both healthy controls and patients subjects. Comparison of the Fwd vs SS condition elicited significant increases in theta power in the right medial prefrontal regions. These results suggest this right frontal theta activity may reflect recruitment of the auditory working memory buffer in maintaining the thread of the ongoing narrative in the healthy controls. The presence of this signal in these two severely brain-injured subjects with high-level language function suggest the possible utility of this method as passive search paradigm for active listening.

Disclosures: H.M. Markell: None. **L.F. Mendels:** None. **M.M. Conte:** None. **N.D. Schiff:** None.

Poster

703. Traumatic Brain Injury: Human Studies

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Topic: C.10. Trauma

Support: NIH 5RO1-HD51912

James S. McDonnell Foundation

Title: Using customized behavioral assessments to measure covert cognition in the severely brain-injured

Authors: L. F. MENDELS[1], T. NAUVEL[1], J. HERSH[2], M. M. CONTE[1], *N. D. SCHIFF[3]; [1]Brain and Mind Res. Inst., [2]Publ. Hlth., [3]Weill Cornell Med. Col., NEW YORK, NY

Abstract: Although the role of neuroimaging in investigating residual awareness after severe brain injury has expanded in recent years, the primary method of diagnosis of disorders of consciousness (DOCs) remains observational behavioral testing. The current gold standard of behavioral measures, the Coma Recovery Scale-R (CRS-R) has been used to assess subtle behaviors indicative of awareness and volition in DOCs (Giacino, 2004). The communication assessment subscale of the CRS-R, however, does not probe cognitive domains such as memory. Broader, more flexible measures are therefore required. The Individualized Qualitative Behavioral Assessment (IQBA), may be appropriate in such cases where standard verbal or gestural communication channels are unavailable (Whyte, 1999). We studied a subject in

minimally conscious state (M, age 23) who sustained a traumatic brain injury with marked brainstem injury secondary to suboccipital hemorrhage and injuries to the cortex, maximal in right orbitofrontal regions. Four years post-injury, the patient's occupational therapist discovered an unusual yes/no communication channel using control of downward movement of the right arm to areas of the body. We describe the customization of an IQBA to assess the patient's cognitive function based on this channel. We used the IQBA to test the reliability of this novel communication channel and to address aspects of underlying cognitive function: autobiographical information, which tested long-term memory, environmental awareness, which tested short-term memory, and simple logic to test reasoning capacity. A total CRS-R score of 5 (maximum score = 23) reflected an absence of motor function. fMRI and EEG motor imagery paradigms demonstrated command following. The patient was tested at various times during the day with the IQBA by several trained assessors over a three-day inpatient visit. Responses to all of the command following conditions were 100% accurate and the overall responses to yes/no questions were 59% accurate. The patient's responses to logic questions were most accurate compared with questions testing both long and short-term memory (67%, 59% and 50%, respectively). Both memory retrieval and encoding of new information depend heavily on medial temporal lobe integrity (Gabrieli, 1997). These results suggest that injuries to the medial temporal-lobe memory system seen in structural imaging may account for the low performance on IQBA items requiring anterograde memory function. The IQBA results reveal aspects of the patient's residual cognitive function and illustrate the value of customized assessments in MCS patients.

Disclosures: L.F. Mendels: None. **J. Hersh:** None. **T. Nauvel:** None. **M.M. Conte:** None. **N.D. Schiff:** None.

Poster

703. Traumatic Brain Injury: Human Studies

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Topic: C.10. Trauma

Support: NIH grant 5R01-HD51912

James S McDonnell Foundation

The Tri-Institutional Program in Computational Biology and Medicine

Title: Test-retest reliability of multitaper quantitative resting state EEG measures in healthy volunteers

Authors: *T. J. NAUVEL, M. M. CONTE, N. D. SCHIFF;
Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: The EEG is a useful diagnostic and research tool in many areas of neuroscience. In recent years, quantitative EEG measures have been used to assess the reliability of test-retest EEG. (Näpflin et al, 2007). In particular, quantitative spectral analysis methods have been shown to be useful in the assessment of abnormal cortical dynamics in patients with disorders of consciousness (Williams et al, 2013, Conte et al, 2010, Drover et al, 2013). Furthermore, other quantitative measures such as global coherence or functional EEG network connectivity measures are also potentially useful diagnostic biomarkers, but their test-retest reliability has not yet been studied. In this study, continuous CCTV-EEG was collected using an augmented 10-20 bipolar montage at two timepoints (at least 6 months apart) from both healthy volunteers and patient subjects. Multiple resting state periods (5 mins minimum) were collected over the course of the day to account for diurnal variations, during which subjects were monitored for alertness (eyes open). For every dataset, we assessed the test-retest reliability of spectral measures. Power spectra and coherence were calculated using multitaper methods as implemented by the Chronux matlab toolbox. Global coherence was calculated using methods from Cimenser et al, 2011. Functional network connectivity measures were calculated based on correlation and coherence measures of the EEG originally described in Chu et al, 2013. We focus here on the test-retest reliability of these quantitative EEG measures in healthy volunteers. Establishing the reliability of spectral features will allow these parameters to provide benchmarks for quantitative and automated methods of assessing changes in the EEG which are not due to recording variability but underlying functional changes in the brain. Here we find that in healthy subjects the stability of these EEG measures within wakefulness show strong reproducibility within and across testing sessions. But these results are highly dependent on the quantity and quality of data collected, as well as the subject's arousal level. We illustrate the use of these measures in analyses obtained from one patient with severe brain injury studied longitudinally who demonstrated measured changes in qEEG associated with late recovery of communication and structural alterations in brain white matter (Thengone et al, 2013). We also demonstrate how these spectral features can be used as parameters in a GLM model (Näpflin et al, 2007) to assess the effect of subcallosal cingulate DBS on depression in a previously reported longitudinal EEG study (Nauvel et al, 2013).

Disclosures: T.J. Nauvel: None. **M.M. Conte:** None. **N.D. Schiff:** None.

Poster

703. Traumatic Brain Injury: Human Studies

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James S McDonnell Foundation Consortium for Recovery of Consciousness

Title: Differential electroencephalographic responses to familiar and unfamiliar music processing in disorders of consciousness

Authors: *B. C. FIDALI, D. J. THENGONE, T. NAUVEL, Z. M. ADAMS, N. D. SCHIFF; Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Recent research has suggested variable levels of covert cognition in patients with disorders of consciousness (DOC) as measured by electroencephalographic (EEG) and functional neuroimaging. This study compares EEG responses to familiar and unfamiliar music in patients across the DOC clinical spectrum. Music stimuli are a powerful tool for measuring cortical activity, as they recruit more bilateral processing areas than does speech during passive listening and isolate aspects of non-dominant hemisphere processing. Previously, minimally conscious patients have demonstrated selective desynchronization and power modulation during the presentation of personally meaningful music. These findings were not observed while listening to unfamiliar music, suggesting active and alert attention (Thengone et al., 2012). Similar modulation of alpha as well as other frequency bands have been demonstrated in response to preferred music in a separate DOC population (O'Kelley et al., 2013). 37 channel CCTV-EEG recordings were obtained in 15 DOC patients admitted for inpatient studies. Music stimuli were epoched into 3-second segments corresponding to the presentation of familiar and unfamiliar music unique to each patient. Music familiarity was based on family/caregiver interviews; one favorite song of the patient heard regularly before cerebral insult was presented along with unfamiliar songs in similar and disparate genres that were produced after their date of injury and not featured on television or radio. We used multitaper estimates of power spectra and statistical validation via taper-based jackknife techniques (significant differences identified by the Two Group Test over a 2 Hz range, for $p = 0.05$ with FDR correction, Chronux MATLAB Toolbox). Eight (3 F, mean age 29) of 15 patients with disorders of consciousness demonstrated differential responses to familiar and unfamiliar music. Power spectra changes were noted in the theta, alpha,

and beta frequency ranges. The specific frequency and spatial distribution over the cortex varied with the functional state and cerebral lesion of the patients, reinforcing the injury-specific patterns of recovery in disorders of consciousness. These data support previous findings of differential EEG responses to passive musical stimuli in DOC and suggest correlates of auditory attention as well as autobiographical and auditory memory in this population.

Disclosures: B.C. Fidali: None. **D.J. Thengone:** None. **T. Nauvel:** None. **Z.M. Adams:** None. **N.D. Schiff:** None.

Poster

703. Traumatic Brain Injury: Human Studies

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

Support: NIH Grant 5R01HD51912

James S. McDonnell Foundation

Title: Normalization of sleep architecture in the setting of central thalamic DBS implantation in a minimally conscious patient

Authors: *Z. M. ADAMS, P. B. FORGACS, D. J. THENGONE, M. M. CONTE, N. D. SCHIFF;
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Abstract: Minimally conscious state (MCS) is characterized by intermittent behavioral awareness of the self and external environmental stimuli. MCS patients typically have abnormal sleep/wake cycles post-injury, however, these abnormal sleep patterns may contain inconsistent elements of normal sleep architecture, and the potential emergence of these sleep elements over time is not well characterized. We used quantitative EEG (qEEG) to analyze the sleep of one MCS patient subject with a traumatic brain injury at three research admission time points beginning 20 years after the initial event: 1 month before implantation of a central thalamic deep brain stimulator (ctDBS), and 3 and 4 years post-ctDBS. At each admission, the CCTV-EEG was continuously recorded (augmented 10-20 montage) and monitored over several days. To analyze this patient's atypical sleep stages, we categorized them into two distinct "stages": (1) a "stage 2-like" sleep that consisted of low amplitude theta-delta waves and spindle-like formations (which

frequently formed in lower-than-normal sleep spindle frequencies), and (2) a “slow-wave sleep (SWS)-like” stage that included predominantly low frequency (delta) waves with occasional higher frequency spindle-like peaks riding on top of the delta waves. Power spectra for each abnormal sleep “stage” were calculated using multitaper methods as implemented in the Chronux matlab toolbox. Of note, this patient subject did not show behavioral improvements at the bedside based on Coma Recovery Scale-Revised (CRS-R) scores across the observed time points (mode total CRS-R score of 12). At the first admission, power spectral analysis showed a peak in the 8.7-9.5 Hz range in the “stage 2-like” stage. A delta peak (1-2 Hz) and an abnormally low frequency spindle-like peak were observed (in the “SWS-like” stage), representing a “mixed” stage (one lower frequency component as well as one higher frequency component in one spectrum). By the second and third admissions, spectral analysis of the “stage 2-like” stage reflected a shift towards a normalized spindle frequency (11-12 Hz), and a spindle peak was no longer present in the “SWS-like” stage. These data suggest a pattern of normalization of spectral features of sleep in an MCS patient who received ctDBS due to the emergence of isolated sleep stages, and increased frequency of spindle-like formations. These results provide insight into the electrophysiological aspects of sleep recovery that may occur post-ctDBS treatment.

Disclosures: Z.M. Adams: None. **P.B. Forgacs:** None. **D.J. Thengone:** None. **M.M. Conte:** None. **N.D. Schiff:** None.

Poster

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Program#/Poster#: 703.07/R3

Topic: C.10. Trauma

Support: James S. McDonnell Foundation

Title: Structural connectivity between the thalamus and fronto-temporal regions predicts level of awareness in disorders of consciousness

Authors: *Z. ZHENG[1], N. REGGENTE[1], E. LUTKENHOFF[1], A. OWEN[2], M. MONTI[1]; [1]UCLA, Los Angeles, CA; [2]Univ. of Western Ontario, London, ON, Canada

Abstract: A reliable neural biomarker would serve as a valuable prognostic indicator for the assessment of awareness in patients with disorders of consciousness (DOC). Previous research has suggested that DOC may reflect disconnections in the thalamocortical networks. In this

current study, we used probabilistic tractography to investigate the structural connectivity between the thalamus and the rest of the brain in 23 patients with varying levels of behavioral awareness as measured by the coma recovery scale-revised (CRS-R). The CRS-R spans six subscales aimed at assessing overt consciousness: auditory, visual, motor, oromotor, communication, and arousal. We obtained a global CRS-R score for each patient by summing across all six-subscale scores, where a maximum total score would be 23 points. We employed a searchlight mapping approach by centering a 5mm sphere at each voxel in the brain. The thalamic-connectivity-index values of voxels within each sphere were used as predictors in a ridge-regression. The predictive power of our model was assessed by a leave-one-patient-out cross-validation whereby we iteratively trained a ridge-regression model on 22 subjects and applied that model to the left-out subject. The resulting vector of predicted CRS-R scores correlated with the actual CRS-R scores most strongly when the searchlight was centered in frontal (medial and middle frontal) and temporal (parahippocampal/hippocampal) regions. More specifically, connections with the right thalamus in these regions accounted for upwards of 45% of the variance in CRS-R scores. Furthermore, inferior frontal and parahippocampal connections with the left thalamus accounted for up to 25% of the variance in CRS-R scores. These results provide neural bases for the level of conscious awareness displayed by DOC patients. More specifically, this investigation highlights the importance of thalamo-prefrontal and thalamo-temporal circuits in establishing a dependable anatomical metric for calculating patients' CRS-R scores. Such findings support the "disconnection syndrome" hypothesis by illustrating that decreases in structural connectivity throughout the brain correlates with degradations in conscious awareness.

Disclosures: Z. Zheng: None. **N. Reggente:** None. **E. Lutkenhoff:** None. **A. Owen:** None. **M. Monti:** None.

Poster

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Topic: C.10. Trauma

Title: Vestibular-cognitive-postural effects and a biomarker signature from sub-concussive head impact

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Abstract: Sub-concussion is an underrecognized phenomenon resulting from low levels of head impact that has the potential to cause significant neurological damage long-term. Soccer heading provides a safe and unique human model to study controlled head impacts. Here we investigated the effect of sub-concussive impacts from soccer heading on behavioral and biomarker measures in a pre-, 0-2hr post-, 24hr post-heading repeated measures design. Ten healthy young adult (ages 18-25) soccer players with at least 5 years of soccer heading experience participated. Soccer balls were projected from a JUGS machine at a speed of 25 mph as subjects performed 10 standing headers over 10-minutes at the beginning of the second test session (0-2hr post-test). Blood samples were immediately taken for subsequent analyses to determine whether biomarker and genetic analysis correlate with individual differences in behavioral measures. During balance testing subjects stood on a foam surface while Galvanic Vestibular Stimulation (GVS) was applied. Gain and phase relative to GVS was calculated. For biomarker detection, blood samples were drawn from a subset of the population (N=7) and collected into Acid-Citrate-Dextrose tubes at each time point. Cell-derived microparticles were assessed by flow cytometry. The standing balance results showed that gains relative to GVS were significantly decreased in 0-2hr post session compared to the pre- and 24h post-session. The decrease and recovery of gain correlated with clinical measures (BESS, signs/symptoms, neurocognitive). The number of microparticles of neurovascular origin was significantly increased (mean: 1.9-fold, range: 1.2 - 3.2 folds) at 24hr post session suggesting potential cerebrovascular damage induced by the sub-concussive episode. These results reflect that mild mechanical insults affect a number of behavioral measures, suggesting that even sub-concussive impact may have detrimental consequences due to long-term repetitive exposure.

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Poster

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Topic: C.10. Trauma

Support: Center of Excellence for Clinical Trial and Research in Neuroscience Grant DOH 101-TD-B-111-003

National Science Council Grant NSC 102-2321-B-038-005

Title: The association of IGF-1 and mental disorders after mild traumatic brain injury

Authors: *Y.-H. CHIANG[1], K.-Y. CHEN[2], C.-C. WU[1], S.-H. TSAI[3], J.-C. OU[4];
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Abstract: Introduction: Mild traumatic brain injury (mTBI) is the one of the major public problems and it results in several physical and psychiatric problems, such as headache, fatigue, sleep disturbance or depression. The Insulin like growth factor 1 (IGF-1) system is involved in growth and survival signaling in the central nervous system. The aim of our study was to determine the association between IGF-1 levels and the severity of mental disorders following mTBI. Methods: 101 mTBI patients and 106 healthy participants (control group) were recruited for our observational study. The severity of mental disorder is surveyed by the number of psychiatric problems, including anxiety measured by Beck anxiety inventory, depression by Beck depression inventory II, daytime sleepiness by Epworth sleeiness scale and sleep quality by Pittsburgh sleep quality index. Mental disorder severity are then divided into five severity groups are no, slight mild, mild, moderate, and severe. The patients' age were divided into there groups, young ,< 31 years old, middle, 31 - 50 years old, and old, >50 years old. The association was evaluation via three-way ANOVA and the Tukey's multiple comparisons of means. Results: The IGF-1 levels of mTBI were significant lower than that of the control group. From the multiple comparisons, the mean IGF-1 level of the young age group was significantly higher than that of both median age and old age group. In addition, the IGF-1 levels of the moderate and severe groups were significantly lower than that of no problem group. Conclusion: Patients after mTBI contain lower IGF-1 levels than the control group. Age is also a factor affecting the IGF-1 levels. Moreover, the mTBI Patients with moderate to severe mental disorder had lower IGF-1 levels than the control group and the patients without any mental disorder.

Disclosures: Y. Chiang: None. **K. Chen:** None. **C. Wu:** None. **S. Tsai:** None. **J. Ou:** None.

Poster

703. Traumatic Brain Injury: Human Studies

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Topic: C.10. Trauma

Support: National Institute for Health Research Professorship Grant (United Kingdom)

Title: Post-traumatic amnesia: Disconnection between the hippocampus and default mode network

Authors: *S. DE SIMONI[1], P. GROVER[1], P. O. JENKINS[1], G. SCOTT[1], M. H. WILSON[2], A. D. WALDMAN[1], D. J. SHARP[1];
[1]Med., [2]Surgery and Cancer, Imperial Col. London, London, United Kingdom

Abstract: Introduction Post-traumatic amnesia (PTA) is very common early after traumatic brain injury (TBI). PTA is characterised by a confused, agitated state and a pronounced deficit in the ability to encode new memories. Its pathophysiology is poorly understood. The hippocampus is central to memory processing, and normally shows strong functional connectivity to nodes within a large-scale intrinsic connectivity network, the default mode network (DMN). We hypothesise that a functional disconnection of the hippocampus from the DMN will be present in patients with acute PTA, with a normalisation of this connectivity accompanying the end of PTA. Methods Functional magnetic resonance imaging (MRI) was acquired from 11 healthy controls, 5 TBI patients without PTA and 5 patients in profound PTA. The presence of memory impairment (i.e. PTA) was assessed with the use of the Paired Associative Learning (PAL) task as part of the Cambridge Neuropsychological Test Automated Battery (CANTAB) computerised tool. Patients were investigated within two weeks of their injury. Two PTA patients were scanned at two timepoints (6 months apart). All data were analysed using FSL. Resting state data preprocessing included realignment of functional images, spatial smoothing using an 8 mm full-width at half-maximum Gaussian kernel. Functional images were registered to standard MNI space using the participant's high- resolution T1. Changes in functional connectivity between a central node of the DMN, the posterior cingulate cortex (PCC), the hippocampus and parahippocampus were assessed using a dual regression approach. Neuropsychological data was analysed using SPSS 21.0. Results At baseline, patients with PTA demonstrated significantly reduced functional connectivity between both the anterior hippocampus and parahippocampus and the PCC, compared to controls and patients without PTA. A significant interaction in functional connectivity was also found between the anterior and posterior hippocampi. At follow-up, the PTA patients who were assessed at two timepoints, showed both normalised memory function and functional connectivity between the hippocampus, parahippocampus and the PCC. Conclusions The results suggest that hippocampal and parahippocampal pathology is involved in the causation of PTA, and that transient functional disconnection between brain regions involved in memory formation, including nodes within the DMN, may underlie the profound cognitive impairments seen in PTA. Different patterns of connectivity between the

PCC and the anterior and posterior hippocampi during PTA support the functional segregation between these two regions.

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Poster

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Topic: C.10. Trauma

Title: Traumatic brain injury prematurely ages the brain

Authors: *J. COLE, R. LEECH, D. J. SHARP;
Computational, Cognitive & Clin. Neuroimaging Lab., Imperial Col. London, London, United Kingdom

Abstract: Background The long-term effects of traumatic brain injury (TBI) can resemble those observed in normal ageing. This poses the question; does TBI hasten brain ageing? Here we use neuroimaging to investigate this question by building a model that predicts brain age in healthy individuals and then applying it to TBI patients. In this way we test for the presence of a discrepancy between chronological age and predicted 'brain age', consistent with accelerated ageing after TBI. Methods In a machine learning framework, Gaussian Processes Regression (GPR) was used to define a predictive model of normal ageing in a sample of 1537 healthy individuals, using T1-MRI estimates of grey matter (GM) and white matter (WM). This ageing model was then applied to a sample of 99 TBI patients and 113 healthy controls to estimate brain age. Findings The initial ageing model predicted age with a high degree of accuracy: the correlation between chronological age and predicted age was r = 0.92. For TBI patients, the mean predicted age difference (PAD) between chronological and estimated brain age was 4·66 years (± 10·8) for GM and 5·97 years (± 11·22) for WM. There was also a strong correlation between PAD score and time since injury, indicating that the amount of premature brain ageing increases throughout the chronic post-injury phase. PAD score also correlated with neuropsychological measures, particularly those related to information processing speed, that are frequently affected in TBI. Interpretation The results indicate that TBI patients' brains are 'older' than their chronological age. Further, suffering a moderate-severe TBI appears to accelerate the rate of brain ageing, because PAD increases with time since injury, as did decline in cognitive

performance. These findings may relate to the increased susceptibility of TBI patients to age-associated conditions such as cognitive impairment and dementia and has implications for possible therapeutic approaches.

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Poster

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Topic: C.10. Trauma

Support: National Science Council Grant NSC 102-2321-B-038-005

Title: Correlation of Bmx/Etk serum level to dizziness and depression indices after mild traumatic brain injury

Authors: *K.-Y. CHEN[1], Y.-H. CHIANG[1,2], C.-C. WU[2], Y.-R. TSAI[1], J.-C. OU[3];
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Abstract: Introduction: Bmx/Etk, a member of the Tec family of nonreceptor tyrosine kinases, was upregulated after traumatic neural injury in rat model. The objective of this investigation was to determine if Bmx/Etk serum concentrations can effectively be used to predict outcome after mild TBI (mTBI). Methods: A total of 104 patients with mTBI (Glasgow Coma Score [GCS] between13-15) were included. Blood samples taken at the time of hospital admission were analyzed for Bmx/Etk. Data collected included demographic and clinical variables. Outcome was assessed using Dizziness Handicap Inventory (DHI) and Beck Depression Inventory II (BDI) questionnaires at 6 weeks post injury. Results: The mean serum level of Bmx/Etk was higher in mTBI patients than healthy control both at baseline and 6th week follow-up. Based on linear regression adjusted by age, gender, and the injury status (brain injury or not), level of depression was higher in women at both baseline and 6th week assessment. However, women had higher level of dizziness at baseline assessment significantly, but not at 6th week. A multiple regression model identified that a baseline Bmx/Etk predicted level of dizziness at baseline, and level of depression at 6th week. As Bmx/Etk increased one unit at baseline assessment, the score of DHI increased 0.61. Similarly, the baseline Bmx/Etk increased one unit as the score of BDI increased 0.21 at the 6th week assessment. Conclusion: Bmx/Etk may be a

useful marker for brain damage in mTBI patients and seems to be associated with the presence of dizziness and depression after the trauma.

Disclosures: **K. Chen:** None. **Y. Chiang:** None. **C. Wu:** None. **Y. Tsai:** None. **J. Ou:** None.

Poster

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Topic: C.10. Trauma

Support: NSC 102-2331-B038-003, Taiwan

Title: Heart rate variability as a prognostic indicator of emotional disorders in patients with mild traumatic brain injury

Authors: ***J. WANG**[1], C.-W. SUNG[2], Y.-H. CHIANG[3], K.-Y. CHEN[3], J. OU[4];
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Abstract: Depression and anxiety are the most frequently diagnosed emotional disorders after mild traumatic brain injury (mTBI); however, predictors of these disorders remain uncertain. The non-invasive measurement of heart rate variability (HRV) has gained popularity as a functional index of the ANS. In this study, we demonstrate that power spectral analysis of HRV at early stage successfully predicts the occurrence of emotional disorders such as anxiety and depression at late stage in mTBI patients. The group studied consisted of mTBI patients and healthy volunteers in our affiliated hospitals. Two important psychological evaluations, Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) were part of a 6- week up to 2-year follow up assessment. Among these patient groups, as well as the volunteer group, we recorded individuals' 5-min resting assessment of HRV. Results show some significant correlations between serum biomarkers (cortisol, melatonin, IGF-1 and serotonin) and HRV parameters in healthy volunteers and mTBI patients. These findings also indicate that mTBI patients, compared to the healthy controls, are more vulnerable to emotional disorders, as evaluated by BAI and BDI scores. These results also have implications as a potential method for predicting whether the mTBI patients are susceptible to emotional disorders using HRV analysis.

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Poster

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Topic: C.10. Trauma

Support: NIH R21 EB006120

NIH P41 EB015909

DOD W81XWH-08-1-0192

Title: Persistence of resting-state fMRI derived outcome measures from weekly sessions over 185 weeks

Authors: *A. S. CHOE[1], S. E. JOEL[1], C. K. JONES[1], J. MUSCHELLI[3], J. W. MCDONALD[2], V. BELEGU[2], P. C. M. VAN ZIJL[1], J. J. PEKAR[1];
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Abstract: Introduction: Existence of a positive autocorrelation, or "persistence," in a dataset indicates that there is a tendency for a system to persist in the same state from one measurement to the next. Understanding of such autocorrelative nature of a system allows better identification significant covariance or correlation between measurements of the system, and allows the design of a more robust predictive model for the observed system (*e.g.,* predictive model of recovery for spinal cord injury patients undergoing rehabilitation). Resting state fMRI (rs-fMRI) can be used to identify brain functional networks without requiring subjects to perform explicit tasks, therefore allowing the use of an identical imaging protocol for all patients regardless of their cognitive or physical limitations. Interest in using the methodology to monitor progress during clinical trials and rehabilitation therapies for chronic conditions such as spinal cord injury and cerebral palsy motivated the present study, which made use of a remarkable longitudinal dataset reporting on one healthy adult subject scanned on a weekly basis over a period of 185 weeks (~ 3 yrs). The dataset provided means to perform time series analysis on the weekly rs-fMRI derived outcome measures obtained over 185 weeks - allowing a unique opportunity to observe the

degree of autocorrelative nature, as well as the intra-subject inter-session reproducibility of the rs-fMRI outcome measures. **Materials and Methods:** A healthy participant underwent an rs-fMRI session weekly at a regular basis for 185 weeks. Preprocessing was performed, followed by principal component and group independent component analysis. Fourteen components were identified as functional networks. Spatial correspondence of single-session maps and the corresponding group mean maps was computed using η^2. Between network connectivity (BNC) was computed as the Pearson correlation coefficient of the network time courses. **Results:** Autocorrelation was observed in both of the outcome measures, with autoregressive AR(1) model explaining time series of many networks most closely. Spatial stability was high, reflected by η^2 values ranging from 0.747 to 0.841. Two visual networks showed the least reproducibility, with coefficient of variation (CV) values of 6.68 and 4.86%, while two executive networks showed the highest reproducibility, with CV values of 1.65 and 1.60%. For BNC, the executive left and right network pair was most reproducible with a CV value of 13.6%. **Conclusions:** GICA derived rs-fMRI outcome measures display persistency, and spatial network maps and BNC are stable over a period of 185 weeks.

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Poster

703. Traumatic Brain Injury: Human Studies

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Topic: C.10. Trauma

Title: Identification of a long-term stress signature in the hippocampus of prenatally stressed rats and in the blood of subjects with childhood trauma history by using mRNA-miRNA integration approach

Authors: *A. CATTANEO[1], A. LUONI[2], C. PARIANTE[1], M. RIVA[2];
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Abstract: Early life exposures to stressful events produces widespread changes on brain function that predispose the individuals to develop a wide range of pathologies later in their life. Although it is still unclear how childhood trauma can induce such vulnerability, it likely involves non-

genetic mechanisms that affect complex network of biological pathways. The identification of biological mechanisms relevant to disease vulnerability could lead to the identification of biomarkers as well as new pharmacological targets for preventive therapies. Emerging evidence suggests that many of these changes might be driven by microRNAs (miRNAs). Evidences suggests that miRNA levels change during stress and are affected in depression (Dwivedi et al., 2011). On these bases, we used the prenatal stress (PNS) model and the blood of healthy controls with a history of childhood trauma to investigate molecular alterations induced by early life adversities that may contribute to the vulnerability for a large spectrum of disorders at adulthood. We performed transcriptomic and miRNome analyses in the hippocampus of adult rats, which have been exposed to stress prenatally with the aim to identify coordinated changes in genes and in miRNAs. We then validated our gene expression findings in the leukocytes of 40 healthy subjects characterized by childhood trauma exposure. The gene expression analyses conducted in the hippocampus of adult rats identified 873 genes and 68 miRNAs significantly regulated by PNS. Thereafter, a meta-analysis for miRNA binding sites was used to detect possible miRNAs binding sites, and only miRNAs with significant genes interactions, were used to carry out further analyses. We thus filtered out 3 up- and 21 down-regulated miRNAs as the most significantly modulated by PNS and also as highly significantly enriched for their binding sites within deregulated genes. A pathway analyses on these selected miRNAs indicated alterations in several biological processes including metabolic, calcium signalling, MAPK, TGFβ and chemokine signalling pathways. We then selected and measured the expression levels of miR-let7a, miR-322, miR-494 and miR-362 in the leukocytes of healthy subjects which reported a history of childhood trauma to validate their role in the maintenance of stress signature following exposure to early life adversities. Our data provide support to the notion that early life stress enhances the vulnerability depression through changes in gene expression that may be set in motion by coordinated disruption of miRNAs. The characterization of these mechanisms may ultimately lead to the identification of novel targets for pharmacological intervention.

Disclosures: **A. Cattaneo:** A. Employment/Salary (full or part-time):; King's College London London, UK; IRCCS Fatebenefratelli Brescia, Italy. **A. Luoni:** A. Employment/Salary (full or part-time):; Center of Neuropharmacology, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano. **C. Pariante:** 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.; Medical Research Council, National Institute of Health Research and other NHS-related funding , Biomedical Research Centre in Mental Health at South London and Maudsley NHS Foundation Trust and King's. **M. Riva:** 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.; Bristol-Myers Squibb and Sunovion. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); Bristol-Myers Squibb, Dainippon Sumitomo Pharma, Eli Lilly, Roche, Servier and Sunovion.

Poster

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Program#/Poster#: 703.16/R12

Topic: C.10. Trauma

Title: Association of deubiquintinating enzyme with traumatic brain injury

Authors: *I. HAN[1], K. BAEK[2];
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Abstract: Object: Deubiquintinating enzymes (DUBs), also known as ubiquitin-specific proteases (USP) are a large group of proteases that cleave ubiquitin from proteins and other molecules and they are known to be part of DNA repair complexes. In humans there are nearly 100 DUB genes. Although recent studies have shown the expression of DUB genes plays an important role in the development of various diseases, the role of DUB genes in traumatic brain injury is unknown. The aim of the study is to investigate the role of DUB genes in patients with traumatic brain injury. Methods : A total of 60 patients with traumatic brain injury were enrolled in this study. The patients are classified into three groups: controls (no head injury, n=20), minor head trauma (n=20), and severe head trauma (n=20) according to Glasgow coma scale. Peripheral bloods were collected from controls and patients. The expression of DUB genes were analyzed in blood samples using RT-PCR expression analysis, two-dimensional gel electrophoresis (2D-GE) and matrix-assisted laser desorption/ionization (MALDI)-time of flight mass spectrometry (TOF MS). We compared the expression of the DUB genes according to between controls and patients. Results : We found the different expression levels of DUB genes in blood samples were significantly higher compared with controls. Conclusion : The DUB genes may be associated with the risk and severity of traumatic brain injury. Our results suggest that the expression of DUB genes may play an important role as a potentially valuable novel biomarker for traumatic brain injury.

Disclosures: I. Han: None. **K. Baek:** None.

Poster

703. Traumatic Brain Injury: Human Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 703.17/S1

Topic: C.10. Trauma

Title: An evaluation of quantitative electroencephalography (QEEG) in relation to serum levels of biomarkers for brain injury in concussed and non-concussed combat zone cohorts

Authors: *W. CARR[1], D. MILHORN[2], J. ELLIOT[3], N. ROGERS[4], R. BRODNICK[5], M. HARRELL[6], K. SCHMID[1], F. TORTELLA[1], D. HACK[2];
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Abstract: Concussion occurs in both civilian and military environments and its diagnosis remains a challenge in both settings when standard of care neuroimaging results are negative. An objective of this study was to evaluate quantitative electroencephalography (QEEG) results in relation to two serum biomarkers of brain injury as aides to the diagnosis of concussion in a military combat environment. This was an observational cohort study with participants stratified into three groups: Concussed Group, Uninjured Control Group, and Injured (non-concussed) Control Group. The study was conducted in Regional Command Southwest, Afghanistan and participants were 184 U.S. military service members (Concussed=40, Uninjured Control=75, Injured Control=69) who provided informed consent and who were able to complete the EEG recording session. A 10-minute EEG recording was acquired approximately 24 hours after injury (or upon enrollment for the Uninjured Control Group); additionally, two blood draws were taken, one within 8 hours of injury (or upon enrollment for the Uninjured Control Group) and a second approximately 24 hours later. Clinical diagnosis data were supplemented with symptom assessment via the Sport Concussion Assessment Tool 2. Twenty-one EEG records were not of sufficient quality for analysis, leaving 163 cases with complete data sets (Concussed=35, Uninjured Control=65, Injured Control=63). Results showed that QEEG analysis did not discriminate Concussed cases from the two Control groups (beta=-0.04, t=-0.55, p=.583) and did not add predictive value for concussion diagnosis beyond that afforded by serum biomarkers (e.g., Ubiquitin C-terminal Hydrolase-L1, beta=0.22, t=3.87, p<.001) or symptomology (e.g., number of reported symptoms, beta=0.22, t=3.87, p<.001). This pattern of results for QEEG is not consistent with some clinical studies using QEEG in civilian settings; however, there are differences across studies in concurrent computerized tomography results and severity of injuries examined. Further research is warranted. Because a combat zone population is unique,

assessment efforts like that reported here are required to evaluate the comparability of findings in civilian medical research to a military theater of operations, especially in domains like concussion that have a relation to blast exposure and other military unique conditions.

Disclosures: W. Carr: None. **D. Milhorn:** None. **J. Elliot:** None. **N. Rogers:** None. **R. Brodnick:** None. **M. Harrell:** None. **K. Schmid:** None. **F. Tortella:** None. **D. Hack:** None.

Poster

703. Traumatic Brain Injury: Human Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 703.18/S2

Topic: C.10. Trauma

Title: Evaluation of the neurological effects of repeated exposure to low-level blast overpressure during U.S. Army explosives training

Authors: *M. L. LOPRESTI[1], W. CARR[1], A. M. YARNELL[1], T. WALILKO[2], R. M. MCCARRON[3];
[1]Psychiatry and Neurosci., Walter Reed Army Inst. of Res., Silver Spring, MD; [2]Applied Res. Associates, Inc., Littleton, CO; [3]NeuroTrauma Dept., Naval Med. Res. Ctr., Silver Spring, MD

Abstract: Repeated exposure to overpressure from controlled low-level blasts is a growing concern among military and law enforcement personnel working with explosives. Anecdotal reports of concussion-like symptomology and preliminary findings from a series of observational studies suggest neurophysiological effects associated with occupational exposure to blast; although, the evidence is inconsistent and the effects, if any, are small. The objective of this study was to evaluate changes in blood-based biomarkers and neurocognitive performance following exposure to blast overpressure. This study enrolled 108 participants during 3 separate data collections at U.S. Army explosive entry training sites. Subjects wore blast pressure sensors and completed testing at the end of each day throughout 2-week long training cycles. Individual blast exposures were continuously recorded throughout the training cycle using helmet mounted blast sensors, which monitored the frequency, magnitude, and duration of each exposure event. Over 2,000 blast overpressure measurements ranging from 0.5 to 12 pounds per square inch (psi) were recorded. Subjects provided demographic and medical histories to include previous head injury and exposures to controlled and uncontrolled blasts. Outcome measures included computer-based neurocognitive testing, balance testing, symptom questionnaires, sleep pattern analysis, and analysis of blood components (e.g. serum-based proteomic biomarkers). Results

from 1 training site indicated a correlation between blast exposure on the first day of close-proximity training and an increase in serum Ubiquitin C-terminal Hydrolase-L1 (UCH-L1) concentration ($n = 42$, $r = 0.33$, $p < .05$). Results of this study contribute to the development of new empirically-based measures and preclinical thresholds for assessing effects of repeated blast exposure on the brain.

Disclosures: M.L. LoPresti: None. **W. Carr:** None. **A.M. Yarnell:** None. **T. Walilko:** None. **R.M. McCarron:** None.

Poster

703. Traumatic Brain Injury: Human Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 703.19/S3

Topic: C.10. Trauma

Support: VA R&D Research Service

NIH T32 AG00258

VA MIRECC Fellowship

Title: Repetitive mild blast in mice induces cerebellar Purkinje cell loss and white matter injury that is anatomically similar to chronic FDG-PET hypometabolism in blast-exposed Veterans

Authors: J. S. MEABON[1], B. R. HUBER[1], E. C. PETRIE[1], D. J. CROSS[3], J. C. WILEY[4], E. R. PESKIND[1], *D. G. COOK[2];
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Abstract: INTRODUCTION: Many Iraq and Afghanistan war Veterans were exposed to multiple explosive blast overpressures (BOPs) and, as a result, suffered multiple mild traumatic brain injuries (mTBIs). We have modeled battlefield-relevant BOP-induced mTBI in mice using a shock tube that accurately recapitulates open-field TNT detonations. In these experiments we focused upon the consequences of mild BOP in the cerebellum. To explore the potential translational significance of this animal model, we compared the distribution and severity of cerebellar neuropathology in blast-exposed mice with that of cerebellar hypometabolism (assessed via brain [18F]FDG-PET imaging) in Veterans with a history of blast exposure. METHODS: Anesthetized 3 month-old male C57Bl/6 mice were exposed to 1 or 3 mild BOPs

(~17 psi, 7 ms; 1 BOP per day) using a helium-driven pneumatic shock tube. At 1 or 30 days post-treatment, confocal microscopy was performed. Using Neurostat 3D-SSP, brain PET images (GE Advance scanner, 7-10 mCi [18F]FDG), were warped to Talairach atlas and Pearson correlations of cerebral glucose metabolism (CMRglu) and log10-transformed blast-mTBI exposures were calculated for each image voxel and r's converted to z-scores, which were evaluated via a random fields model to control Type I error at p=0.05 (Z≥4.0). RESULTS: Mild BOP caused cerebellar injury that was focused primarily in the lower cerebellar lobules as evidenced by extravasation of blood-borne 10 kDa Dextran into the cerebellar cortical parenchyma. At 30 days post-exposure, BOP-exposed mice displayed increased astrogliosis in the cerebellar white matter tracks that was greatest in repetitively exposed animals (GFAP immunostaining [sham vs. blast]: 1 BOP, 1.00±0.04 vs. 1.39±0.9, p≤0.01; 3 BOP 1.00±0.04 vs. 1.69±0.1, p≤0.05). Repetitive, but not single mild BOP also resulted in significant Purkinje cell loss of the inferior cerebellum (p≤0.05) and reduced RotoRod performance (p≤0.05). Resting CMRglu was negatively correlated with log10blast exposures in both cerebellar VOIs (r's= -0.454 & -0.473, p= 0.005 & 0.003, respectively [one-tailed]). In voxelwise analyses, negative correlations were evident in the right inferior semilunar lobule/lobule VIIB (Z's=4.02, 4.09 & 4.37) and right pyramis/lobule VIIIB (Z's=4.38, 4.52 & 4.75). CONCLUSION: Inferior cerebellar injury in mice exposed to repetitive mild BOP and inferior/posterior cerebellar hypometabolism in Veterans with repetitive blast-mTBI exposure are consistent with previous autopsy findings in boxers with chronic traumatic encephalopathy and suggest selective inferior/posterior cerebellar vulnerability to repetitive mild trauma.

Disclosures: J.S. Meabon: None. **B.R. Huber:** None. **E.C. Petrie:** None. **D.J. Cross:** None. **J.C. Wiley:** A. Employment/Salary (full or part-time):; GE Healthcare. **E.R. Peskind:** None. **D.G. Cook:** None.

Poster

703. Traumatic Brain Injury: Human Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 703.20/S4

Topic: C.10. Trauma

Support: DoD TCN 12056

Title: Omic profiling identifies distinct genetic, protein and lipid biomarker profiles correlating with MRI in military service members with TBI and PTSD

Authors: *T. E. EMMERICH[1,2,3], F. CRAWFORD[1,2,3], L. ABDULLAH[1,3], J. EVANS[1,3], G. CRYNEN[1,2], A. HART[1], A. GONZALEZ[1], T. DENNEYJR.[4], J. ROBINSON[4], J. KATZ[4], G. DESHPANDE[4], M. DRETSCH[5,6], J. REED[1,3], M. MULLAN[1,3,2];
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Abstract: Mild traumatic brain injury (mTBI) is a major problem for both military and civilian populations, with long-term sequelae including neurological and neuropsychological dysfunction. To complicate matters, there is a high co-morbidity of posttraumatic stress disorder (PTSD) in military populations. Therefore, identifying approaches for improving diagnostic accuracy and individualizing treatments is necessary due to pattern of overlapping symptoms. Progressive magnetic resonance imaging (MRI) techniques have led to advances in understanding mTBI-related changes in both cerebral structure and function, and allows for assessment of specific neural networks and regions of the brain that appear to be implicated in soldiers with long-term mTBI sequelae. An objective panel of biomarkers for TBI and relevant conditions such as PTSD, linked to the functional and structural markers with both self-report and neuropsychological functioning, would improve triage and appropriate medical management, highlight ongoing pathogenic processes, provide guidance in therapeutic development, and could be used to assess outcomes and response to treatment. In our cross-sectional study a cohort of 157 active duty soldiers provided blood samples for biomarker profiling and were screened for mTBI, psychological health, PTSD, personality traits, and relevant symptoms. Neurocognitive function was assessed using CNS-Vital Signs® and neuroimaging used resting state functional MRI and diffusion tensor imaging (DTI). Clinical subgroups of mTBI, PTSD, and co-morbid cases were analyzed and compared to healthy matched controls. The omics platform utilized liquid chromatography/mass spectrometry (LC/MS) lipidomic analyses, and LC/MS quantitative proteomic analyses was done using tandem mass tag (TMT) labeling for relative and absolute quantitation. Potential genetic influences such as APOE, BDNF and DRD2 were also examined. Our analysis of deployment related biomarkers showed that certain combinations of protein/lipid and genetic markers correlated with the psychometric MRI measures. The findings provide validation for this multimodal approach and may be useful for predicting and diagnosing mTBI , PTSD, and other underlying psychological health conditions. Given the complexity of mTBI and PTSD pathogenesis and the heterogeneity in human populations, a valid biomarker panel using omic approaches for broad application is anticipated.

Disclosures: T.E. Emmerich: None. **F. Crawford:** None. **L. Abdullah:** None. **J. Evans:** None. **G. Crynen:** None. **A. Hart:** None. **A. Gonzalez:** None. **T. DenneyJr.:** None. **J. Robinson:** None. **J. Katz:** None. **G. Deshpande:** None. **M. Dretsch:** None. **J. Reed:** None. **M. Mullan:** None.

Poster

703. Traumatic Brain Injury: Human Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 703.21/S5

Topic: C.10. Trauma

Title: Serum prolactin concentration progressed from lower to higher quartiles in athletes following sports-related concussion

Authors: *M. LAFOUNTAINE[1], A. TESTA[1], W. A. BAUMAN[2];
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Abstract: Following an acute concussive head trauma, a significant outflow of neurotransmitters and metabolites has been demonstrated to occur, with an ultimate effect of cortical excitation. The clinical manifestations of these cellular changes in the acute post-injury period are otherwise unavailable in humans and, as such, require indirect evidence from end-organs or systems that are sufficiently sensitive to the cellular excitation. Dopamine is a neurotransmitter with numerous targets in the central and peripheral nervous systems, with changes to central dopaminergic tone resulting in reciprocal responses of hormones including prolactin (PRL). Thus, a concussive head trauma may lead to abnormal dopaminergic tone resulting in dynamic PRL concentrations in the circulation. To determine the effects of concussion on concentrations of serum PRL, venipuncture was performed between 8 and 11 am in 5 male intercollegiate athletes (age: 20±1 years; height: 71±5 inches; weight: 174±21 pounds) within 48 hours of concussion injury (Visit 1) and again 7 days post-injury (Visit 2; Note: athletes were not taking any medications during the study). After processing by a commercial laboratory, PRL concentrations (ng/ml) were categorized by quartile (Q) for each visit: Q1 (<5 ng/ml); Q2 (5.1-7 ng/ml); Q3 (7.1-11 ng/ml); and Q4 (11.1-34.9 ng/ml). On Visit 1, PRL concentrations for 2 athletes were in Q1 (3.4±1.4 ng/ml), 1 athlete was in Q2 (5.8 ng/ml) and 2 athletes were in Q3 (8.5±1.2 ng/ml). On Visit 2, PRL concentrations for the 3 athletes previously in Q1 and 2 were now in Q3 (9.3±1.8 ng/ml) and the remaining 2 athletes were in Q4 (14.7±13.5 ng/ml). The athlete with the lowest PRL concentration (2.4 ng/ml) on Visit 1 reported complaints of sexual dysfunction and loss of libido, symptoms consistent with hypoprolactinemia that resolved as PRL concentrations increased. The athlete with the highest serum PRL concentration (9.0 ng/ml) on Visit 1 was symptom (i.e., cognitive and somatic) free within 2 days of concussion and cleared for participation in sports; at Visit 2, his PRL concentration was the highest (17.2 ng/ml) value of any of the 5 concussed athletes and was more consistent with reported concentrations in male athletes. Serum PRL concentrations increased and transitioned through higher quartiles 7

days after sports-related concussion. A transient augmentation of central dopaminergic tone would have resulted in a greater inhibition of PRL secretion early after concussion and relaxation may have resulted in disinhibition of PRL release later. This novel observation provides evidence for dopaminergic dysfunction after concussion and may be tracked clinically through serum PRL.

Disclosures: **M. Lafountaine:** None. **A. Testa:** None. **W.A. Bauman:** None.

Poster

704. Spinal Cord Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 704.01/S6

Topic: C.10. Trauma

Support: NIH NS36350 to XMX

NIH NS52290 to XMX

NIH NS50243 to XMX

NIH F31 NS071863 to CLW

Title: *In vivo* and *in vitro* mechanisms of bisperoxovanadium-mediated neuroprotection following traumatic spinal injury

Authors: *C. L. WALKER, X. WU, N.-K. LIU, X.-M. XU;
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Abstract: We previously identified neurons as potential targets of bisperoxovanadium (bpV), a PTEN inhibitor that induced significant neuron survival following cervical hemi-contusion SCI. Although significant reduction in Akt phosphorylation around the injury site was observed 1d post-SCI, it is unclear whether this response is sustained in spared spinal neurons. We also did not confirm whether bpV(pic) acted directly on neuron survival or through PI3K/Akt signaling specifically in neurons. We therefore aimed to assess Akt phosphorylation following SCI, extend our understanding of bpV(pic)-mediated neuroprotection, and effectively determine whether bpV(pic) acts on PTEN and PI3K/Akt signaling in spinal neurons following traumatic injury. Female Sprague-Dawley (SD) rats were utilized. Anesthetized rats received laminectomy or unilateral cervical (C5) SCI via the MASCIS Impactor (10g weight, 12.5 mm height). For *in*

vitro assessment of bpV effects, primary spinal cord neurons were obtained from E15 rat spinal cords. A scratch injury model mechanically damaged 7-10 DIV mixed spinal neuron culture. Pre-treatment with 100 nM PTEN inhibitor bpV(pic), 20 μM PI3K-inhibitor LY294002, 25 nM rapamycin, or combinations of the above were applied. Cells were then cultured for designated time periods. Supernatant was collected for cell death assays and cells were either prepared for Western blot analysis or immunocytochemistry. Lactase dehydrogenase (LDH) assay and Hoechst 33342/propidium iodide (PI) were used to assess cell death. Cells were incubated with AlexaFluor 488-conjugated β-III-tubulin primary antibody for neuronal labeling. PTEN activity, as a ratio of total PTEN to phospho-PTENser380, significantly increased by 3d post-SCI (71.4% increase; $p < 0.05$). p-Akt decreased significantly by 1d (39.1% decrease). SCI significantly increased caspase 3 activity (67.1%) 1d post-injury, and bpV(pic) significantly reduced its activity (27.3%) ($p < 0.05$). *In vitro*, acute LDH release was decreased by 100 nM bpV(pic) post-scratch injury($p < 0.05$). Western blot determined bpV(pic) increased Akt and S6 protein phosphorylation ($p < 0.05$). Our findings support bpV-mediated neuroprotective effects observed after SCI, and this study is the first to demonstrate bpV effects on survival and PTEN/PI3K signaling in primary spinal neurons following traumatic injury. In addition, our results show bpV(pic) stimulated Akt/mTOR activity, which suggests potential for not only neuroprotection but also regeneration following SCI.

Disclosures: C.L. Walker: None. **X. Wu:** None. **N. Liu:** None. **X. Xu:** None.

Poster

704. Spinal Cord Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 704.02/S7

Topic: C.10. Trauma

Support: R01N5020013

R01N5020778

Title: Defining the role of β1-integrin in gliosis after spinal cord injury

Authors: S. JEONG[1], *J. A. KESSLER[1], L. PAN[2], H. NORTH[2], T. MCGUIRE[2];
[1]Davee Dept Neurol, Northwestern Univ., CHICAGO, IL; [2]Northwestern Univ., Chicago, IL

Abstract: The objective of this proposal is to investigate mechanisms mediating glial lineage commitment and glial scar formation after spinal cord injury (SCI). In the early phases after SCI, astrocytic hypertrophy facilitates recovery by inhibiting infiltration of inflammatory cells and helping to seal the blood brain barrier. However astrocytic hyperplasia later leads to formation of a dense scar that inhibits axon regrowth. We have shown that injection of self-assembling peptide amphiphile (PA) with isoleucine-lysine- valine-alanine-valine (IKVAV) epitope into the lesion decreases glial scar formation and leads to improved motor function after SCI. IKVAV is known to exert is biological action by engaging b1-integrin (b1-int). However, the molecular mechanism of action is unknown. Ependymal stem cells (ESCs) in the spinal cord generate the majority of new astrocytes after SCI, and ESCs abundantly express b1-int and that ablation of ablation of b1-int from cultured neural stem/progenitor cells promotes astrocytic differentiation. These observations suggest that IKVAV PA regulates glial scar formation by altering b1-int signaling in ESCs. Glial lineage commitment is also regulated by bone morphogenetic protein (BMP) signaling. BMPs are extracellular signaling molecules that exert their effects by binding to a transmembrane receptor consisting of a tetramer of two type I (BMPRIa or BMPRIb) and two type II (BMPRII) receptor subunits. BMPRIa and BMPRIb signaling may exert differing and even opposing effects in the nervous system. For example, we have shown that BMPRIa signaling regulates the initial astrocytic hypertrophic response after SCI whereas BMPRIb signaling facilitates the later hyperplastic response. We have recently demonstrated that b1-int and BMP receptors interact directly, and that ablation of b1-int in cultured neural progenitor cells (NPCs) derived from the subventricular zone (SVZ) increases trafficking of BMPRs into lipid rafts. Further, we find that ablation of b1-int increases BMP signaling (levels of nuclear phospho-SMAD 1/5/8 and phospho-p38) in cultured NPCs and increases astrocytic lineage commitment. This suggests that interactions between b1-int and BMP signaling pathways may regulate astrogliogenesis after SCI. Therefore, we hypothesize that b1-int modulates BMP signaling by regulating the trafficking of BMP receptor (BMPR) subunits into lipid rafts, and that this interaction regulates astrocytic differentiation of ependymal stem cells and glial scar formation after spinal cord injury.

Disclosures: S. Jeong: None. **J.A. Kessler:** None. **L. Pan:** None. **H. North:** None. **T. McGuire:** None.

Poster

704. Spinal Cord Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 704.03/S8

Topic: C.13. Sensory Disorders

Title: Treatment with diluted bee venom of the ST36 acupoint reduces both spinal inflammatory responses and central neuropathic pain behaviors after spinal cord injury in rats

Authors: *S.-Y. KANG[1], D.-H. ROH[2], J.-H. LEE[3], O. KWON[1], S. YEON[1], K.-H. CHOI[1], S. CHO[1], S. CHOI[1], Y. RYU[1];
[1]Acupuncture Moxibustion, Korea Inst. of Oriental Med., Daejeon, Korea, Republic of; [2]Kyung Hee Univ., Seoul, Korea, Republic of; [3]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Chemical acupuncture with diluted bee venom (DBV) has been traditionally used in eastern medicine to treat several inflammatory diseases or chronic pain conditions. We have previously shown that DBV had a potent anti-inflammatory and anti-nociceptive efficacy in several rodent pain models. In the present study, we investigated whether the treatment of DBV into Zusanli (ST36) acupoint suppressed intraspinal inflammatory responses as well as allodynic and hyperalgesic behaviors in the spinal cord injury (SCI) model of rats. SCI was induced by T13 spinal cord hemisection after laminectomy. SCI surgery produced acute migration of the neutrophils and the dramatic increment of myeloperoxidase (MPO) activity in the spinal cord lesions at 24 hours following hemisection. In addition, the mechanical allodynic and thermal hyperalgesic behaviors were developed in the bilateral hind paws throughout the 28 days of experiment. Subcutaneous injection (0.25 mg/kg) of DBV was applied into Zusanli acupoint twice a day for five days. DBV treatment significantly suppressed neutrophils infiltration and the MPO activity at 24 hours after hemisection. Moreover, mechanical allodynia and thermal hyperalgesia were relieved throughout the experimental period. DBV injection also showed the facilitated motor function recovery as indicated by the Basso-Beattie-Bresnahan rating score. Finally, spinal glial fibrillary acidic protein (GFAP) expression, a marker for astroglial activation, was also suppressed by DBV injection.These results demonstrated that the repetitive application of DBV into acupoint not only enhanced functional recovery but also reduced acute-inflammatory response and neuropathic pain behavior after SCI. This study suggests that DBV acupuncture can be a potential clinical therapy for management of SCI.

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Poster

704. Spinal Cord Injury

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 704.04/S9

Topic: C.10. Trauma

Support: NYS SCIRB - C020931

SUNY HSC Foundation

Title: Pattern of oligodendrocyte progenitor cell migration following a spinal contusion injury

Authors: *J. R. SIEBERT[1], K. H. FLYNN[2], K. SWIECK[2], D. J. OSTERHOUT[2];
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Abstract: A major barrier to spinal cord repair is the appearance of a glial scar formed after a spinal cord injury. This scar is largely composed of chondroitin sulfate proteoglycans (CSPGs) which are expressed at high levels very quickly following injury. CSPGs have long been known to be inhibitors to axonal sprouting and regenerative growth, but only recently found to be inhibitory to oligodendrocytes. The expression of CSPGs has also recently been found to inhibit the migration of endogenous oligodendrocyte progenitor cells (OPCs) that reside in the adult spinal cord and are recruited to site of tissue damage. Migration of OPCs is inhibited by the presence of specific CSPGs, such as neurocan, which is expressed at high levels in and around the lesion center. CSPGs are not only upregulated at the site of injury, but at levels distal to the initial site of injury. Increased levels of CSPGs have been observed at at the cervical and lumbar enlargements after a thoracic contusion injury. This suggests that CSPGs can inhibit repair processes both at the initial site of injury and at distal spinal segments. In this study we examined the effect of CSPG upregulation on the migration of endogenous OPCs to a spinal cord lesion from spinal levels both rostral and caudal to the site of moderate spinal contusion injury. Previous work has demonstrated that resident OPCs will migrate to the edges of a spinal lesion. A preliminary analysis shows that there are a greater number of OPCs that appear at the rostral edge compared with caudal edge. Examination of areas further away from the lesion, including cervical and lumbosacral spinal enlargements, show a similar pattern, with more cells appearing in the cervical regions than in the lumbar regions. This pattern of migration may correlate with the levels of proteoglycans in the lumbar and cervical enlargements. Inhibitory CSPGs such as neurocan are elevated not only in the lesion center but in spinal segments further away from the lesion. This data suggests that molecular changes in the spinal cord at regions distal from the lesion may affect the capacity for repair and regeneration.

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Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: P30 GM103507-04 (SRW, JC)

Pilot Grant of Pediatric Department (JC)

Title: Functional recovery following spinal cord injury in platelet-activating factor receptor null mutant mice

Authors: *Y. WANG[1,2], Z. GAO[1,2], Y. ZHANG[3], R. WU[2], Q. ZHU[4], D. GOZAL[5], C. B. SHIELDS[3], J. CAI[2];
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Abstract: Introduction: Reactive gliosis is characterized by the proliferation of microglial cells and astrocytes as well as astrocytic hypertrophy. However, the molecular switch control of astrocytic state and its precise inhibitory mechanism to axonal regrowth remains poorly understood. Platelet activating factor (PAF) is a unique phosphoglycerine that mediates the biological functions of both immune and nervous systems. Excessive PAF appears to play an important role in neural injury via its specific receptor (PAFR). In this study, we hypothesized that PAF signaling activates the reactive gliosis after SCI and blocking of PAF pathway would modify the formation of glial scar and promote functional recovery. **Methods**: 8-week old wild-type C57BL/6 and PAFR null mutant mice were conducted a dorsal hemisection at C5-6 spinal level with 0.75 mm depth and 1.00 mm width using the Vibraknife, which cut off entire dorsal columns (ascending somatosensory tracts) and partial corticospinal tracts. The functional recovery was evaluated by use of grip and accelerating rotarod tests at 3, 7, 14, 21, 28, 35 and 42 days post injury (dpi). The spinal tissues at C5-6 level were collected at the same time points. Activated microglia/macrophage marker CD68, astrocyte markers GFAP and nestin, inflammatory cytokines IL-6, and ECM components (CSPG) were examined by immunohistochemistry and/or West blot. Furthermore, the PAFR competitive antagonist Ginkgolide B were injected subcutaneously (100mg/kg body weight) once a day in the wild-type SCI mice before and after 10 dpi respectively, and functional recovery were assessed till 42 dpi. The statistical significance was considered as $p<0.05$. **Results**: 8-week old adult PAFR null mutant mice showed normal development of microglias/astrocytes and expression of IL-6. In the

wild-type SCI mice, expressions of GFAP and nestin were elevated consistently between 3 dpi and 42 dpi, but not changed significantly in PAFR null injured mutants. Disruption of PAF signaling also inhibited the expressions of proteoglycan CS56 and neurocan (aka CSPG3) and improved both grip and rotarod performance. More intriguingly, late-stage but not early-stage antagonist treatment enhanced the functional recovery in the wild-type SCI mice. **Conclusion**: These findings suggest that PAF signaling may participate in the reactive gliosis and ECM remodeling after SCI.

Disclosures: Y. Wang: None. **Z. Gao:** None. **Y. Zhang:** None. **R. Wu:** None. **Q. Zhu:** None. **D. Gozal:** None. **C.B. Shields:** None. **J. Cai:** None.

Poster

704. Spinal Cord Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 704.06/S11

Topic: C.10. Trauma

Title: Analysis of metabolic profile of the first 7-days following spinal cord injury using a porcine model of SCI

Authors: *E. B. OKON[1], F. STREIJGER[1], J. H. T. LEE[1], N. MANOUCHEHRI[1], K. SO[1], B. KWON[1,2,3];
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Abstract: Characterization of the basic metabolic responses to acute traumatic spinal cord injury (SCI) may help in understanding how different injury mechanisms influence secondary damage after SCI. Recently, we developed a technique to collect microdialysis samples from the parenchyma of the injured spinal cord in pigs following contusion SCI with or without compression. We demonstrated that in the first few hours after injury, disturbances in energy metabolism within the injured spinal cord vary greatly depending upon the biomechanical nature of the injury. To expand upon these findings, we prolonged the evaluation period to examine these metabolic responses for up to 7-days post-injury using our porcine model of SCI. Microdialysis probes were placed in the exposed spinal cord of Yucatan minipigs at a distance of 2 cm and 4 cm caudally from the projected impact site. Subcutaneously implanted perfusion pumps were used to infuse perfusion fluid through the microdialysis probes at a flow rate of 0.5

microliters/min. Spinal cord injury was induced by a 50-g weight drop from a 50-cm height followed by one hour of compression with 150 g. Dialysates were collected every 15-30 minutes for the first 10 hours after SCI, after which sampling continued twice daily for up to 7-days. Microdialysis samples were analyzed for lactate, pyruvate, glucose, glutamate and glycerol using the ISCUSFlex analyzer. The spinal cords were harvested, processed and stained with Eriochrome Cyanine to visualize the damage induced by the impact and the probe placement. Spinal cord contusion followed by 1-hour of compression resulted in an immediate increase in lactate, L/P ratio (mostly due to a pronounce decline in pyruvate), glutamate and glycerol levels close to the impact site (2 cm). During the compression period, glucose levels dropped significantly. Decompression of the cord restored glucose levels and decreased L/P ratio. During the post-surgery recovery period, lactate, pyruvate, and L/P ratio levels remained slightly increased for up to 7-days compared to SHAM-control levels, while glycerol gradually decreased. The metabolic alterations at the 4 cm position were less pronounced. In conclusion, in the acute phase (hours) of SCI the metabolic disturbances in the tissue around the injury site revealed significant changes in the expression of metabolic markers related to tissue damage, ischemia and excitotoxicity. The metabolic perturbations in the tissue were evident (L/P ratio) for up to 1 week post-injury, however markers of tissue damage and excitotoxicity subsided at 7-days post-injury.

Disclosures: **E.B. Okon:** None. **F. Streijger:** None. **J.H.T. Lee:** None. **N. Manouchehri:** None. **K. So:** None. **B. Kwon:** None.

Poster

704. Spinal Cord Injury

Location: Halls A-C

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Topic: C.10. Trauma

Support: Norwegian Research Council

Southeastern Norway Health Authority

Medical Faculty, University of Oslo

Title: Characterization of cellular reactions during adaptive plasticity after a spinal cord injury in the neonatal mouse

Authors: *R. S. CHAWLA[1], M. ZÜCHNER[1,2], C. B. SYLTE[1], J. C. GLOVER[1,3,4], J.-L. BOULLAND[1,3];
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Abstract: An important phenomenon that occurs following spinal cord injury in rodents is adaptive plasticity, wherein spinal networks spontaneously form novel connections that contribute to functional recovery. If adaptive plasticity can be harnessed (and maladaptive plasticity avoided), it could provide a new platform for clinical treatment. We have recently shown that neonatal spinal cord injured (SCI) mice exhibit more functional recovery than reported in adult SCI mice, and demonstrated that during recovery novel synaptic connections are formed from decending pathways to spinal MNs (Boulland et al 2013). Here we have begun to characterize the cellular reactions that occur during behavioral recovery. We have focused on cell proliferation, synaptic density, and target neuron number. Methods: A thoracic spinal cord compression was performed with a modified aneurysm clip. EdU was injected i.p. daily from 1 to 8 days post-injury. L1 and L2 MNs were labeled retrogradely and counted or stained for presynaptic terminals using anti-synaptophysin-1. Specific cell types were identified by immunolabelling with NeuN, ChAT, GFAP and Iba1, and EdU+ proportions determined. Results: 8 days post injury there is a virtually complete loss of neurons at the compression epicenter, and a reduction of neuron number by a factor of 3 within the more distal parts of the compressed segment. Despite this dramatic loss, there was about a 30% increase in the number of EdU+ cells compared to controls, and these included GFAP+ and Iba1+ cells (putative astrocytes and microglia, respectively). There was a 6-fold reduction in synaptic terminals on lumbar MNs 1 day post-injury, but this normalized by 8 days post-injury. During this time, there was no change in the number of lumbar MNs as a consequence of thoracic injury. We conclude that behavioral recovery in the neonatal mouse following thoracic compression injury is due primarily to the regainment of lost synapses, probably paralleled by a reorganization of synaptic connections, and not on compensatory neurogenesis. We are now focusing on similar analyses of neuronal number and synaptic connectivity within defined interneuron populations.

Disclosures: R.S. Chawla: None. **M. Züchner:** None. **C.B. Sylte:** None. **J.C. Glover:** None. **J. Boulland:** None.

Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

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NINDS R21NS082835

DOD W81XWH131007715

Miami Project to Cure Paralysis

Buoniconti Fund

Title: Hematogenous macrophages assemble the fibrotic scar after spinal cord injury

Authors: *Y. ZHU, C. SODERBLOM, J. ZHA, V. KRISHNAN, J. ASHBAUGH, J. BETHEA, J. K. LEE;
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Abstract: After spinal cord injury (SCI), a scar tissue forms at the injury site. This scar tissue is comprised of an astroglial portion that surrounds the injury site and a fibrotic component that occupies the lesion epicenter. While recent studies have shown that the astroglial scar sometimes can be growth permissive for axon regeneration, the fibrotic scar is considered to be largely inhibitory. The fibrotic scar is best characterized by the presence of perivascular fibroblasts and a fibronectin matrix. However, the mechanism by which perivascular fibroblasts are recruited to the injury site or how the fibronectin matrix is formed is unknown. Using transgenic mice in which fibroblasts are labeled with green fluorescent protein and an adoptive transfer approach, we discover that fibroblasts and hematogenous macrophages are the major cellular components of the fibrotic scar and both of them express integrin receptor α5β1, a canonical receptor for fibronectin. Whereas hematogenous macrophages fill up the fibrotic scar, activated microglia are mostly restricted to the astroglial scar. By administering liposome-encapsulated clodronate, we depleted hematogenous macrophages which results in reduced fibroblast number and increased axon growth in the fibrotic scar. Together, our results underscore the important roles of hematogenous macrophages in formation of the fibrotic scar and provide a potential cellular target to reduce the scar formation and enhance axon regeneration after SCI.

Disclosures: Y. Zhu: None. **C. Soderblom:** None. **J. Zha:** None. **V. Krishnan:** None. **J. Ashbaugh:** None. **J. Bethea:** None. **J.K. Lee:** None.

Poster

704. Spinal Cord Injury

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Program#/Poster#: 704.09/T2

Topic: C.10. Trauma

Support: Canadian Institute of Health

Multiple Sclerosis Society of Canada

Title: PDGFRα-positive progenitor cells form myelinating oligodendrocytes and Schwann cells following contusion spinal cord injury

Authors: ***P. L. ASSINCK**, G. J. DUNCAN, J. R. PLEMEL, M. J. LEE, J. LIU, W. TETZLAFF;
ICORD/University of British Columbia, Vancouver, BC, Canada

Abstract: Contusive spinal cord injury (SCI) results in considerable demyelination of spared axons, which impairs signal transduction and may leave axons vulnerable to degeneration. Both oligodendrocytes (OLs) and Schwann cells remyelinate denuded axons in the subsequent weeks and months following SCI. NG2 cells, characterized by the near ubiquitous co-expression of platelet derived growth factor receptor α (PDGFRα) in the uninjured central nervous system (CNS), are oligodendrocyte progenitors (OP)s which serve as a source of new OLs following SCI. PDGFRα-CreERT mice were crossed with Rosa26-YFP mice and administered tamoxifen to label OPs two weeks prior to contusive thoracic SCI. In the uninjured spinal cord, we found that YFP was expressed in NG2+ OPs at very high efficiency, as well as in vascular associated cells and fibronectin+ fibrocytic cells in the spinal roots. Following injury, many recombined cells continue to express PDGFRα, Olig2 and NG2, indicating that they remained as OPs. In addition, substantial differentiation into new CC1+ mature oligodendrocytes was observed, particularly in the spared ventral and lateral white matter. Strikingly, the majority of P0+ Schwann cells in the spinal cord expressed YFP, suggesting they originated from central nervous system PDGFRα+ OPs. However, further work is required to characterize if other YFP+ populations like vascular associated cells or the peripheral fibrocytic-like cells can contribute to the formation of myelinating Schwann cells or OLs in the injured CNS. Overall, we reveal enormous phenotypic plasticity of PDGFRα precursors. Following SCI, these cells are a source of new remyelinating Schwann cells and oligodendrocytes.

Disclosures: P.L. Assinck: None. **G.J. Duncan:** None. **J.R. Plemel:** None. **M.J. Lee:** None. **J. Liu:** None. **W. Tetzlaff:** None.

Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: 1RO1NS074882-01A1

1F31NS080512-01

Title: Activity-Dependent myeloid trafficking in locomotor networks after thoracic SCI: Impact on neuroplasticity

Authors: *C. N. HANSEN[1], S. KERR[2], L. FISHER[1], R. DEIBERT[1], E. WOHLEB[1], J. GODBOUT[3], J. SHERIDAN[4], D. BASSO[2];
[2]Sch. of Hlth. and Rehabil. Sci., [3]Dept. of Neurosci., [4]Div. of Biosci., [1]The Ohio State Univ., Columbus, OH

Abstract: Spinal cord injury (SCI) initiates inflammatory signaling along the neuroaxis that jeopardizes plasticity and function. While much work has characterized neuroinflammation at the lesion epicenter, much less is understood regarding inflammation in remote lumbar locomotor networks. We previously reported that the active gelatinase, matrix metalloproteinase-9 (MMP-9) extends at least 9 segments caudal to a midthoracic injury in the lumbar enlargement and impedes exercise-based recovery (Hansen et al., 2013). It is unclear if remote production of MMP-9 facilitates peripheral recruitment of myeloid progenitor cells (MPCs) into remote networks. MPCs respond to stress-induced sympathetic outflow in the bone marrow and may be primed by exercise to generate neurotoxicity. Thus, in a series of mouse experiments, we characterized neurovascular reactivity and MPC trafficking in the lumbar cord after T9 SCI. To describe trafficking in remote lumbar segments, we quantified protein (ELISA) and immunohistochemistry changes in C57BL/6 (WT) mice (n=17). Increased ICAM-1 identified vascular adhesion and endothelial reactivity within 24h that was maintained throughout the first week after SCI ($p<0.05$). Increased CCL2 signaling that initiates immune cell diapedesis occurred at 24h ($p<0.05$) and returned to baseline by 7d. To determine if myeloid cells infiltrate the lumbar enlargement and/or influence stability of the vascular barrier after T9 SCI, GFP+ bone marrow (BM)-chimeric mice were used (n=22) alongside assessments of vascular permeability in WT mice (n=29). Indeed, peripheral myeloid trafficking occurred within 24h at least 13 segments away from the lesion throughout lumbar and sacral gray matter ($p<0.05$). Consequently, vascular breakdown occurred throughout lumbosacral segments by 7d. Treadmill training reduced peripheral circulation of CD11b+/Ly6C^{hi} cells and differentially regulated their deposition in lumbar central pattern generator laminae. Current work is underway to examine

dendritic spine morphologies in relation to MMP-9 mediated immune cell deposition using Thy1+GFP-BM-RFP and MMP-9-null-BM-RFP chimeras (n=40). Together, our work identifies novel inflammatory mechanisms that occur remote to the lesion around locomotor networks and jeopardize neuroplasticity in an activity-dependent manner. Therapeutic strategies that target remote myeloid trafficking may enhance locomotor plasticity to promote motor relearning and recovery.

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Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: NINDS R01NS081040

NINDS R21NS082835

DOD W81XWH131007715

Miami Project Buoniconti Fund

Title: NG2 cell fate after contusive spinal cord injury

Authors: *A. HACKETT, D. LEE, A. DAWOOD, P. TSOULFAS, K. K. PARK, J. K. LEE; The Miami Project To Cure Paralysis, Miami, FL

Abstract: Although commonly known as oligodendrocyte progenitors, NG2 cells have been shown to display lineage plasticity in several models of central nervous system injury. However, the fate of NG2 cells after the contusive spinal cord injury is still not known. Using NG2-CreER transgenic mice bred to Rosa26-Tdtomato reporter mice, we performed genetic fate mapping after a T8 contusive spinal cord injury. NG2 cells show a six-fold increase in number at the injury site at both 1 and 4 weeks post injury. NG2 cells were densely populated around the injury site in the region of the astroglial scar. Approximately 12% of the fate-mapped NG2 cells coexpressed GFAP at the injury site, whereas there was no coexpression in the uninjured spinal

cord. These results suggest that resident NG2 cells react to SCI and that a subpopulation of NG2 cells become astrocytes after spinal cord injury.

Disclosures: **A. Hackett:** None. **D. Lee:** None. **A. Dawood:** None. **P. Tsoulfas:** None. **K.K. Park:** None. **J.K. Lee:** None.

Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: Great Lakes Colleges Association, New Directions Initiative

Marine Biological Laboratory (MBL) Whitman Center

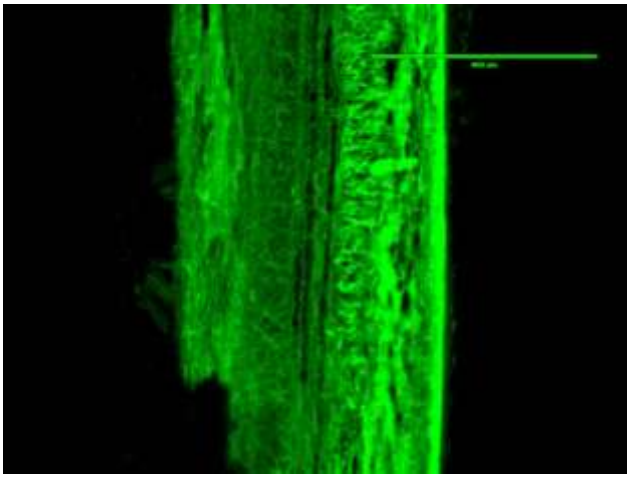
Earlham College Ford/Knight Endowment, Stephenson Fund, Professional Development Fund

Title: Decreased sodium channel expression during spinal cord regeneration in lamprey

Authors: J. A. JURAS[1], A. A. KURUP[1], R. Y. LEWIS[1], R. C. PALMARINI[1], E. S. RICHARDS[1], Y. J. RODRIGUEZ[2], *R. L. ROSENBERG[3];
[1]Biochem., [2]Neurosci., [3]Biol., Earlham Col., Richmond, IN

Abstract: There are ~273,000 people living with spinal cord injury (SCI) in the US today, with ~12,000 new cases per year. Decreased quality of life, low chances of full recovery, decreased life expectancy, and health care costs of up to $1 million/person/year make SCI a devastating condition. Lampreys are a well-characterized vertebrate model for SCI. Unlike higher vertebrates, lampreys exhibit spinal cord regeneration, allowing them to swim almost normally 10-12 weeks after complete spinal transection. New knowledge on lamprey spinal cord regeneration could help identify targets and mechanisms for improved recovery from SCI in humans. Voltage-gated sodium channels (NaV) are ion channels that allow neurons to create and propagate action potentials. Although NaV are required for neuronal function, excessive NaV activity after injury could cause hyper-excitability and excitotoxicity that can kill neurons. Lampreys recovering from SCI are resistant to NaV blockers, suggesting that the expression of NaV is decreased. Thus, changes in NaV expression may be involved in the survival and regeneration of spinal neurons following SCI. This study aims to assess NaV expression in

uninjured and transected lamprey spinal cords. We use immunofluorescence microscopy of transverse and longitudinal spinal cord sections to visualize NaV in spinal cords. To quantify expression, we use image analysis of immunofluorescence micrographs and Western blots. We also perform behavioral studies to measure swimming ability and anesthesia susceptibility during recovery. Our preliminary data provide evidence of decreased expression of voltage-gated sodium channels in regenerating lamprey axons.



Disclosures: **J.A. Juras:** None. **A.A. Kurup:** None. **R.L. Rosenberg:** None. **R.C. Palmarini:** None. **R.Y. Lewis:** None. **E.S. Richards:** None. **Y.J. Rodriguez:** None.

Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: NIH grant P30 GM103507

PVA Research Foundation grant 2934

Title: Pam3-CSK4-mediated neuroprotective mechanisms in spinal cord injury

Authors: ***S. OKADA**[1], N. STIVERS[1], D. P. STIRLING[1,2];
[1]Neurolog. Surgery, [2]Microbiology and Immunol., Univ. of Louisville, Louisville, KY

Abstract: Toll-like receptor 2 (TLR2) promotes locomotor recovery following spinal cord injury (SCI). Although the role of TLR2 in inducting and modulating inflammatory responses is well established, the cellular and/or molecular mechanisms underlying TLR2's neuroprotective effect in SCI remains unclear. We hypothesized that stimulation of the TLR2 pathway after SCI could alternatively activate macrophage/microglia and consequently modulate their typical pro-inflammatory responses in order to improve neurological outcomes. In this study, we investigated the effects of Pam3-CSK4, a synthetic TLR2-specific agonist, on axonal retraction and secondary axonal degeneration; macrophage and microglia immune functions; and locomotor recovery after SCI. Using two-photon microscopy with an *ex vivo* laser-induced SCI (LiSCI) model, myelinated axonal responses with Pam3-CSK4 or control were evaluated in real-time after SCI. Microglia and macrophage population subsets following a T9/10 moderate contusion SCI were identified and the production of iNOS, arginase, and pro-/anti-inflammatory intracellular cytokines were evaluated via flow cytometry. Finally, neurological recovery outcomes were assessed with the Basso Mouse Scale. We found after LiSCI, Pam3-CSK4 treatment reduced retraction/dieback of axons proximal to the lesion and decreased secondary axonal degeneration. After contusion SCI, our flow cytometry results indicated Pam3-CSK4 did not affect the predominant pro-inflammatory M1 macrophage response but inducible nitric oxide synthase (iNOS) and interleukin 6 (IL-6) production were significantly decreased compared to vehicle control. Using the same pro-inflammatory M1- and anti-inflammatory M2-associated macrophage markers, we found that the major microglia population subset responding to the injury exhibited a M2-like phenotype and were functionally more heterogeneous than their M2 macrophage counterparts. M2-like microglia lacked IL-6 but they expressed iNOS and/or arginase. Similar to M1 macrophages, Pam3-CSK4 significantly decreased iNOS production in M2-like microglia. Finally, Pam3-CSK4-treated mice showed greater functional recovery following SCI compared to control-treated mice. Overall, our data indicates that Pam3-CSK4 may improve neurological recovery after SCI by reducing axonal injury as well as moderating inflammatory responses by macrophages and microglia.

Disclosures: **S. Okada:** None. **N. Stivers:** None. **D.P. Stirling:** None.

Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: Wings for Life Spinal Cord Research Foundation (WFL-US-004/12)

Title: Acidic laminin injection for spinal cord repair

Authors: *A. E. HAGGERTY[1], T. L. NOVOSAT[2], M. OUDEGA[2];
[1]Bioengineering, [2]Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Following spinal cord injury (SCI), poor endogenous regeneration of damaged axons may limit the restoration of disrupted circuitries and function. Developing therapies that regenerate axons after injury would enhance the chances for functional recovery. A number of growth-promoting approaches have been explored, but none identified as an effective therapeutic for SCI. Laminin is a key component of the basement membrane and involved in axon growth and guidance in the developing central nervous system. Previously it was shown that laminin polymerized at pH4 (acidic laminin, aLam) is structurally different from laminin polymerized at pH7 (neural laminin, nLam) and promotes outgrowth from immature cortical neurons. We have previously found growth promoting effects of aLam and nLam on axon growth of adult sensory neurons *in vitro* and regeneration and limited early recovery of function *in vivo* in a peripheral nerve crush model. In the current study, we consider the potential of aLam for repair after SCI. Using an adult rat model of contusive SCI, we investigated the potential of aLam to elicit repair. Axonal tracing and immunocytochemistry were used to evaluate anatomical repair. Various motor and sensory tests were employed to evaluate functional recovery. The current results do not reveal anatomical or functional improvements. Future experiments will need to optimize the dose and treatment protocol for aLam to maximize its reparative competence for the injured spinal cord.

Disclosures: A.E. Haggerty: None. **T.L. Novosat:** None. **M. Oudega:** None.

Poster

704. Spinal Cord Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

Support: NS 061973

Title: The role for alarmins in neuroprotection after CNS injury

Authors: *S. GADANI, J. WALSH, J. KIPNIS;
Univ. of Virginia, Charlottesville, VA

Abstract: The role for alarmins in neuroprotection after CNS injury Authors: Sachin Gadani, Jamie Walsh, Igor Smirnov, Jonathan Kipnis Spinal cord trauma is a devastating injury, frequently resulting in hemi/paraplegia with little prospect for healing over time. The contribution of inflammation to recovery remains controversial, with the conventional wisdom that inflammation is purely detrimental increasingly being challenged by studies showing beneficial effects of immune cells and molecules. In this study we focus on the role of CNS derived alarm signals in initiating and amplifying the immune response to injury. We have observed an unexpectedly high expression of molecular alarmins in myelinated tissues, notably the optic nerve and spinal cord, which co-localizes with oligodendrocyte lineage ($olig2^{+}$) cells. Furthermore, elimination of molecular alarmin signaling results in worse outcome after both spinal cord and optic nerve injury. The poor outcome of mice lacking alarmin signaling is associated with severely impaired recruitment of $ly6c^{hi}$ monocytes to the site of injury. In addition to previously described beneficial immunomodulatory affects, these cells are important phagocytes, clearing apoptotic and myelin debris which impede recovery. Interestingly, early released alarmins potentiate phagocytosis of macrophages *in vitro*, suggesting an additional roles for them in promoting clearance after CNS trauma. These studies provide mechanistic insight into the signals that initiate a beneficial immune response in CNS injury and could help guide future immunomodulatory therapies in CNS injury.

Disclosures: S. Gadani: None. **J. Walsh:** None. **J. kipnis:** None.

Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: Wings for Life

EU FP-6 Network of Excellence NeuroNE

EU FP-7 project Plasticise

Title: Manipulation of chondroitin sulphates in regulating regeneration and plasticity in the CNS

Authors: J. C. F. KWOK, *J. W. FAWCETT;
Ctr. Brain Repair, Cambridge Univ., Cambridge, United Kingdom

Abstract: Up-regulation of chondroitin sulphate proteoglycans (CSPGs) in the lesion area is a hallmark for central nervous system (CNS) injury. The inhibitory nature of CSPGs results in the inhibition of neuronal regeneration. Unselective removal of the chondroitin sulphate (CS) chains using chondroitinase ABC has successfully restored functional recovery by enhancing regeneration and plasticity. However, recent evidence suggests that various sulphated isoforms of CS may function differently in the CNS, and some may even result in an enhancement of regeneration if calibrated properly. Using a transgenic mouse model with a depletion of chondroitin 6-sulphates (C6ST-1 KO) by knocking down the synthetic enzyme chondroitin 6-sulphotransferase 1 (C6ST-1), we observed a reduction in axonal re-growth after injury in the CNS when compared to wild-type littermate controls. The importance of 6-sulphation is further strengthened when re-growth and functional recovery is found normal in the peripheral nervous system where there is a compensatory up-regulation of C6ST-2 to replenish the 6-sulphated CS. This suggests that the presence of 6-sulphation is beneficial to basal regeneration and contributed positively to axon regeneration in both CNS and PNS. Recently, we have demonstrated that CS sulphation in the perineuronal nets, a structure present on neuronal surface and is involved in controlling plasticity, regulates the binding and thus the presentation of matrix molecules to neurons. Manipulation of the sulphations modulates the presentation of these molecules which may ultimately lead to a change in plasticity. The results here highlight the importance of CS sulphations in regulating regeneration and plasticity in the CNS.

Disclosures: J.C.F. Kwok: None. **J.W. Fawcett:** None.

Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: the Division of Intramural Research of the National Heart, Lung, and Blood Institute of NIH.

Title: Functional implications of plasticity-related gene-3 (PRG-3) in neuronal cells

Authors: *P. YU[1], C. AGBAEGBU[2], D. A. MALIDE[3], Y. KATAGIRI[1], H. M. GELLER[1]; [1]Developmental Neurobio. Section, NHLBI, NIH, Bethesda, MD; [2]Dept. of Physics,, Georgetown Univ., Washington DC, DC; [3]Light Microscopy Core Facility, NHLBI, NIH, Bethesda, MD

Abstract: The six-transmembrane protein PRG-3 belongs to a protein family called plasticity-related gene (PRG-1 to -5), which were recently identified as a novel brain-specific subclass of the lipid phosphate phosphatase (LPP) superfamily. PRGs share homology with LPP but with no detectable lipid phosphatase activity due to non-conservative substitution in the catalytic motif. We previously used a proteomics approach that identified PRG 3 phosphorylation as being regulated by CSPGs, a major component of glial scar that prevent axon regeneration after central nervous system (CNS) injury. PRG-3 was reported to induce filopodia and neurite protrusions in several cell lines as well as primary neurons. However, the mechanism for these effects and whether PRG-3 is involved in axon regeneration remain unknown. We found that PRG-3 overexpression in primary neurons promotes neurite outgrowth, while knock down of PRG-3 using siRNA inhibits neurite outgrowth. More importantly, overexpressing PRG-3 could also overcome the neurite growth inhibition of CSPGs and this effect was greatly attenuated by deleting the C-terminal portion of PRG-3 which contains multiple phosphorylation sites. In order to better understand the function and underlying mechanisms of PRG-3, we used co-immunoprecipitation followed by mass spectrometry-based proteomics analysis to identify the binding proteins of PRG 3 in neuroblastoma cell line Neuro2A. Overall, we identified hundreds of protein candidates involved in various biological processes as potential binding partners of PRG-3. One of the most intriguing findings was that the other three PRG family members PRG-1, PRG-2 and PRG-5 were found associated with PRG-3.We also revealed a novel role of PRG-5 in facilitating the trafficking of PRG-3 and other PRG family members from the intracellular membrane systems to the plasma membrane, which resulted in an increased induction of protrusions. Another novel finding was we have identified mammalian target of rapamycin (mTOR) and phosphatase and tensin homolog (PTEN) associated with PRG-3. PTEN and mTOR have been shown to play pivotal roles in regulating neuronal intrinsic regenerative ability after CNS injury. Revealing the interaction of PRG-3 with PTEN and mTOR further implies a possible role of PRG-3 in the regulation of regenerative axon growth.

Disclosures: P. Yu: None. **C. Agbaegbu:** None. **D.A. Malide:** None. **Y. Katagiri:** None. **H.M. Geller:** None.

Poster

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Shriners Hospitals for the Children

Craig H. Neilsen Foundation 280274

Title: Dorsal root axons terminate regeneration by forming synapses with NG2 glia

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Abstract: Using the first *in vivo* imaging analysis (Di Maio et al., J. Neurosci, 2011), we previously found that dorsal root axons rapidly terminate regeneration at the dorsal root entry zone (DREZ) by forming numerous axon swellings with the features of presynaptic boutons. These observations led us to support the idea that the regeneration failure might be due to aberrant synaptogenesis with incorrect targets, which causes growth to cease prematurely at the DREZ. To test this hypothesis, we are examining the possibility that the unknown postsynaptic cells in contact with presynaptic boutons are NG2 glia (i.e., oligodendrocyte precursor cells (OPCs)), which form functional synapses with various CNS neurons. We have found that NG2 glia and their numerous thin processes are abundant in the CNS territory of the DREZ, and that they rapidly proliferate in response to distant injury of dorsal roots forming an intense 'cellular net' where axons usually terminate regeneration. Almost all of the axon tips and shafts intensely labeled with synapse markers are either co-localized or in close apposition with processes of NG2 glia. We have also cultured NG2+ OPC cells from adult mice and co-cultured them with adult DRG neurons. At many of the cellular contacts between co-cultured NG2 glia and DRG axons, axon endings or branches exhibit intense immunoreactivity for synapse or active zone makers, suggesting that DRG axons form synaptic contacts with NG2 glia *in vitro*. We plan to carry out electrophysiological analysis and *in vivo* ablation of NG2 glia. Our results thus far support the notion that NG2 glia at the DREZ form a previously unappreciated barrier to nerve regeneration.

Disclosures: H. Kim: None. **S. Han:** None. **G. Thomas:** None. **G.M. Smith:** None. **S.H. Kang:** None. **Y. Son:** None.

Poster

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Shriners Hospitals for the Children

Title: Constitutively active ErbB2 prolongs activation of Schwann cells and promotes peripheral but not intraspinal regeneration of dorsal root axons

Authors: Y.-J. SON[1], S. HAN[1], *H. KIM[2], R. PARK[1], S. KIM[1], S. LI[1], G. M. SMITH[1], A. SKUBA[1];
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Abstract: Dorsal root (DR) axons fail to regenerate through the dorsal root entry zone (DREZ), the CNS/ PNS border where Schwann cells interface with CNS glial cells. Why axons stop at the DREZ remains unclear; one idea to explain the regeneration failure is that Schwann cells, a potent growth supporter, also fail to penetrate the DREZ and lead axon regeneration. In the present study, we tested whether enhanced or prolonged activation of Schwann cells can promote intraspinal migration of Schwann cells and/or axonal regeneration across the DREZ. To this end, we used S100-rtTA/TetO-NeuNT mice in which expression of a mutant, constitutively active neuregulin receptor (caErbB2), can be induced selectively in Schwann cells by a Tet-On system. We have found that, when expression of caErbB2 is induced by doxycycline, Schwann cells in the denervated dorsal roots rapidly proliferate and remain dedifferentiated despite contact with regenerating axons. Axon regeneration along the root is markedly enhanced as indicated by much thicker dorsal roots with far more numerous regenerating axons than control roots. Nonetheless, most axons and Schwann cells fail to penetrate the DREZ and many axons turn around at the DREZ and extend back along the peripheral root in association with Schwann cells. Additional removal of chondroitin sulfate proteoglycans (CSPGs) by injecting lentiviruses expressing chondroitinase did not induce intraspinal regeneration of axons and Schwann cells. Behavioral analyses also indicated no functional recovery. We conclude that prolonged activation of Schwann cells markedly enhances peripheral but not central regeneration of dorsal root axons.

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Poster

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Title: Genetically-accessible lesion conditioned axon regeneration in *C. elegans* is independent of the Dual Leucine Zipper Kinase/DLK-1 and mediated by CaMKII/UNC-43

Authors: *C. V. GABEL[1,3], J. SHAY[2], S. CHUNG[1];
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Abstract: Remarkably, some mammalian neurons can strongly regenerate their central axons following a "conditioning" lesion on their peripheral sensory axons. Despite decades of research, this regenerative effect remains poorly understood. Employing subcellular-resolution femtosecond laser ablation we demonstrate a strong lesion conditioning effect in *C. elegans*, where lesion of the sensory dendrite of amphid neurons triggers targeted regeneration of transected axons. We find that this lesion-dependent regeneration readily occurs in Dual Leucine Zipper Kinase/DLK-1 mutant animals that otherwise display no axon regeneration. Similar to mammalian lesion conditioning, pharmacological or genetic disruption of the L-type voltage gated calcium channels stimulates this regeneration in *C. elegans*. Likewise, fluorescent calcium imaging post surgery demonstrates a reduction in sensory activity following dendrite lesions but no such reduction from an axon lesion alone. We find a link between *C. elegans* lesion-conditioned regeneration and ectopic axon outgrowth observed in the same sensory neurons. Activity-reducing genetic mutations that cause ectopic outgrowth, including those specifically disrupting cyclic nucleotide mediated sensory transduction, also stimulate lesion-conditioned axon regeneration without a dendrite lesion. Finally, as with ectopic outgrowth, we find that lesion conditioned axon regeneration is mediated by CaMKII/UNC-43 activity, potentially linking it to pathways modulating developmental neurite outgrowth. Our work demonstrates direct genetic, molecular, and cellular links between three types of axon outgrowth: *C. elegans* lesion conditioning, *C. elegans* ectopic outgrowth, and mammalian lesion conditioning. These findings establish *C. elegans* as a novel model system for the study of lesion conditioning and are defining specific molecular pathways that modulate it. The distinct advantages of *C. elegans*

for genetic tractability as well as *in vivo* imaging, laser surgery, and analysis are facilitating rapid progress towards illuminating a novel pathway underlying the nervous system's intrinsic regenerative capabilities.

Disclosures: C.V. Gabel: None. **S. Chung:** None. **J. Shay:** None.

Poster

704. Spinal Cord Injury

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Support: TWU Department of Biology

The Southeast Missouri State University Department of Physics and Engineering Physics

TWU Research Enhancement Program

Title: Caveolae mediated endocytosis of nanospheres in neuronal cells

Authors: *S. SEBASTIAN[1], T. MCALLISTER[2], S. GHOSH[2], D. HYNDS[1];
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Abstract: Development of novel nano drug delivery systems to encourage axon regeneration and guidance is promising for functional recovery from CNS injury and damage. Biocompatible nanospheres have the potential to target therapeutics to damaged neurons in central nervous system. Difference in surface functionalization will be a key factor in targeting nanospheres to different subcellular destinations. In the present study, B35 and PC12 cells were treated with surface functionalized nanospheres (SFNPs) to learn their mechanism of endocytosis. The nanospheres were surface functionalized with fluorescently tagged -COOH (~750 nm and ~144 nm) and -NH_2 (~150 nm) groups. B35 and PC12 cells were treated with 3 ul of SFNPs for different time intervals starting from 0 minute to 4 hours. Subsequently, GFP-BacMam 2.0 Cell Light reagents were used to label early and late endosomes as well as lysosomes and immunocytochemistry for caveolin was performed. Treatment of PC12 cells with -NH_2 SFNPs demonstrated endocytosis through caveolin coated vesicles. Moreover, within 15 minutes of treatment -NH_2 SFNPs began to endocytose through caveolin coated endosomes. In future experiments, inhibitors of caveolin mediated endocytic pathway and drug loaded nanospheres

will be used to asses endocytosis, drug transport and axonal guidance in mouse corticospinal tract neurons.

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Poster

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CA1060A11

Title: Critical role for protease activated receptor 2-driven interleukin-6 signaling in astrogliosis

Authors: *M. RADULOVIC[1], H. YOON[2], I. SCARISBRICK[1,2];
[1]Neurobio. of Dis. Program, Mayo Grad. Sch., Rochester, MN; [2]Physical Med. & Rehabilitation, Rehabil. Med. Res. Ctr., Mayo Clin., Rochester, MN

Abstract: Reactive astrogliosis is a key component of central nervous system (CNS) injury and disease. Whereas early stages of astrogliosis appear beneficial and initiate wound healing, chronically reactive astrocytes contribute to glial scar formation hindering neural regeneration. Despite its central importance, the underlying molecular mechanisms of astrogliosis are incompletely understood. We recently demonstrated that neurosin (kallikrein 6), a CNS endogenous secreted serine protease, is induced in reactive astrocytes and exhibits prolonged expression in astroglial scar tissue in cases of human spinal cord trauma, multiple sclerosis and glioblastoma multiforme in addition to animal models of these disorders. In the current study, we address the hypothesis that neurosin participates in astrogliosis by proteolytic cleavage and activation of a G-protein coupled receptor, protease activated receptor 2 (PAR2). First, we show that like neurosin, PAR2 is elevated at sites of CNS injury, including contusion compression injury of the murine spinal cord. Furthermore, we show that the ability of recombinant neurosin to drive key hallmarks of astrogliosis, including a rapid transformation of primary murine astrocytes from an epithelioid to a stellate morphology *in vitro*, secretion of the pro-inflammatory cytokine interleukin 6 (IL-6) and increased expression of glial fibrillary acidic

protein (GFAP) are significantly reduced in astrocytes derived from PAR2 knockout mice. Neurosin also promoted increased levels of an astroglial-associated transcription factor, signal transducer and activator of transcription (STAT3). Notably, genetic deletion of PAR2 or pharmacologic blockade of STAT3 signaling using Stattic, significantly reduced neurosin-mediated IL-6 secretion in primary astrocytes. Moreover, genetic deletion of PAR2 was associated with reductions in molecular signatures of astrogliosis in the case of murine SCI, including decreases in GFAP and in Th1 pro-inflammatory cytokines, such as IL-6. Together, these results indicate that the neurosin-PAR2 signaling axis plays essential roles in promoting astrogliosis and therefore should be investigated as a potential therapeutic target to modulate the astroglial scar in cases of CNS injury and disease.

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Poster

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The Southeast Missouri State University Department of Physics and Engineering Physics

Title: Neuronal uptake of surface functionalized nanocarriers by clathrin mediated endocytosis

Authors: *R. AMMASSAM VEETTIL[1], D. HYNDS[1], S. GHOSH[2], T. MCALLISTER[2]; [1]Biol., Texas Woman's Univ., Denton, TX; [2]Dept. of Physics and Engin. Physics, Southeast Missouri State Univ., Cape Girardeau, MT

Abstract: Degenerative or traumatic damage to the central nervous system causes acute neuronal death and the inability of damaged neurons to regenerate their axon leads to persistent loss of function. Nanomaterial-based drug delivery systems provide potential for axon regeneration from specific neurons by crossing blood brain barrier. We have constructed a system that allows targeting through the potential to attach peptides to carboxyl or amino functional groups on the nanocarrier. These nanocarriers are readily endocytosed by B35 and PC12 cells, with -NH2 surface functionalized nanocarriers being localized perinuclearly and-COOH functionalized

nanocarriers localizing mainly to the cytoplasm. It is important to know the cellular uptake mechanism of these systems for effective drug targeting and delivering. In the present study, we analyze the mechanisms of cellular uptake of these surface functionalized nanocarriers. We used fluorophore labeled -COOH and -NH2 surface functionalized nanocarriers to study the mechanism of cellular uptake in B35 and PC12 cells. We used vesicle markers for early endosomes, late endosomes and lysosomes and clathrin immunocytochemistry with confocal imaging to track the endocytotic pathway from 0 to 4 hours after exposure to nanocarriers. We found that both the -NH2 and -COOH surface functionalized nanocarriers were internalized through clathrin mediated endocytosis in both cell lines. In future experiments, we will investigate the mechanisms of cellular uptake and targeting in corticospinal tract neurons. Together, we expect that these results will test the feasibility of functionalized nanocarriers for targeted drug delivery to encourage axon regeneration following nervous system damage. [Supported by TWU Department of Biology, The Southeast Missouri State University Department of Physics and Engineering Physics, and grants from the TWU Research Enhancement Program]

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Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

Support: Craig H Neilsen Foundation

Cardinal Hill

Kentucky Spinal Cord and Head Injury Research Trust

Title: Age-related neuroinflammatory responses in spinal cord injury

Authors: *B. ZHANG[1], W. BAILEY[1], K. BRAUN[1], J. GENSEL[1,2];
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Abstract: The incidence of spinal cord injury (SCI) among older individuals has increased in recent years. According to the Kentucky Injury Prevention and Research Center, in 2007, 61% of all non-fatal SCIs were sustained by individuals >45 years old. In order to understand the impact of receiving an SCI at an older age, we compared locomotor and anatomical recovery in 4 month-old (4 MO ~ 20 year humans) and 14-month-old (14 MO~ 45 human years) mice after sham or mild thoracic contusion SCI (50 Kdyne Infinite Horizons). We hypothesized that age will have a negative impact on repair and recovery after SCI. By 3 days post injury (dpi), there were significant differences in functional deficits between 4 MO and 14 MO mice that remained for 28 days as measured by the BMS locomotor scale, grid walk, and automated treadmill analysis (DigiGait). In addition, anatomical measures of repair were worse in 14 MO animals; 4 weeks after injury the lesion length was significantly longer and there was significantly less spared tissue compared to 4 MO mice. Very few (<4) published studies have examined the mechanisms behind age-related differences after SCI. Macrophages are a hallmark of CNS trauma and can facilitate repair or pathology in the injured spinal cord. Age is a key regulator of macrophage function and aging is associated with increased activation of pathological macrophage phenotypes. Therefore, we hypothesize that age-related differences in the macrophage response may contribute to worse recovery in 14 MO animals after SCI. Using a comprehensive gene array to phenotype different macrophage populations, we found a different patterns of macrophage activation between 14 MO and 4 MO animals. Specifically, 14 MO showed increased activation of pro-inflammatory macrophages and dampened activation of pro-reparative macrophage phenotypes. Collectively, these data demonstrate an important role for age in changes of inflammatory responses and functional recovery in the context of SCI. Most clinical therapies are being examined in individuals regardless of age and are based upon data generated almost exclusively using young animals. Our data highlight the potential for immunomodulatory therapies to have decreased efficacy in aged individual receiving SCI and highlight the need to elucidate the cellular mechanisms contributing to age-related differences in functional recovery.

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Poster

704. Spinal Cord Injury

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Topic: C.10. Trauma

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Veterans Administration

Adelson Medical Research Foundation

Title: Adult myelin stimulates neurite outgrowth from neural progenitor cells

Authors: *G. H. POPLAWSKI[1], R. LIE[1], P. LU[2], C. GEOFFROY[1], B. ZHENG[1], G. COPPOLA[3], D. GESCHWIND[3], M. TUSZYNSKI[1,2];
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Abstract: Adult central nervous system white matter tissue strongly inhibits neurite outgrowth from adult neurons. However, grafts of multipotent neural precursor cells exhibit remarkably extensive axonal elongation through adult white matter when grafted *in vivo* (Lu et al. Cell 2012), suggesting either that early stage neurons are not inhibited by adult white matter, or are actually stimulated by white matter. To address these possibilities, we cultured multipotent neural progenitor cells from E12 mouse spinal cords on crude adult myelin extracts *in vitro* and compared findings to adult DRG neurons. Whereas adult DRG neurons exhibited the predicted 60% reduction in neurite outgrowth on myelin compared to laminin substrates ($p < 0.001$), neurites extending neuronal progenitor cell cultures exhibited a significant 86% increase in neurite length compared to cells cultured in the absence of myelin (laminin substrate; $p < 0.001$). While adult neurite growth inhibition is mediated in part by interactions with Nogo receptors, we found no change in neurite outgrowth when neural progenitor cells were plated on Nogo-deficient myelin, suggesting that the inhibitory and stimulatory pathways of myelin may be mediated by distinct molecules. We are currently investigating differences in the transcriptome of neural progenitors cultured on myelin-containing vs. myelin-free substrates. Collectively, these findings indicate that at least a portion of the ability of multipotent neural progenitor cell grafts to extend very long axons on adult myelin may result from facilitation of growth on this substrate. Insight into mechanisms underlying this effect may generate novel strategies for promoting adult axonal regeneration through myelin.

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Poster

705. Peripheral and Central Nerve Injury

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Title: Functional analysis of the regenerated inferior alveolar nerve after local administration of anti-BDNF antibody to the transected site

Authors: *H. YOSHIKAWA[1], Y. M. VALVERDE[2], T. MAEDA[3], M. KUROSE[4], K. YAMAMURA[4], K. SEO[5];
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Abstract: Introduction: The inferior alveolar nerve (IAN) is the mandibular division of the trigeminal nerve and is often injured by some dental treatments. Clinically, damage to this peripheral nerve may result in chronic intractable pain of the jaw. Based on surgeries of the injured IAN, neuroma is thought to be a possible cause for this post traumatic pain. Our previous morphological findings of animal experiments also have shown that the administration of anti-BDNF antibody inhibits neuroma formation. This encouraged us to study whether morphologically regenerated IAN after local anti-BDNF administration is functional. Therefore, this study aimed to investigate the effects of local anti-BDNF antibody on the mechanical touch threshold as a behavioral response and the jaw opening reflex as an electrically evoked response. Furthermore, we aimed to determine the presence of BDNF at the transected site after nerve injury. **Methods:** IAN was transected in adult Sprague Dawley rats under general anesthesia. The animals were subsequently administered (locally to the injured site) anti-BDNF antibody at 1 μg/μL(AB1) or 10 μg/μL (AB10), or normal saline (NS). Rats were assessed 2-3 weeks after for (1) the withdrawal threshold to mechanical touch to the mental area, (2) the jaw opening reflex (JOR; the threshold and latency of electrically stimulated mental nerve to contract the digastric muscle), and (3) the level of BDNF mRNA (via real-time PCR) in the transection site and the trigeminal ganglion. **Results**: In the NS group, the withdrawal threshold elevated 2-3 days after the transection, and decreased to the level below pre-transection. However, the threshold in AB1 and AB10 rats was transiently increased. The threshold value in the AB1 group was significantly ($p < 0.01$) lower compared with the AB10 group at 7 and 14 days after the transection. In the results of JOR, no significant differences in the threshold and latency were noted. Expression of BDNF mRNA was significantly increased compared with pre-transection and the 24 hours after the transection in the local transected site and the trigeminal ganglion. These results indicate that BDNF is produced by nerve damage in the injured site and the trigeminal ganglion. **Conclusion:** Neutralizing intrinsic BDNF may inhibit neuroma formation

but not the functional recovery. Supported by grants of MEXT/Japan Society for the Promotion of Science KAKENHI: No's. 23390461/25463132.

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Poster

705. Peripheral and Central Nerve Injury

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Support: NS083377-01 National Institutes of Health/ National Institutes of Neurological Disease and Stroke

Title: Spectroscopic evidence for lower neuronal metabolism and time-dependent increases in inhibition within the former sensorimotor hand territory of chronic unilateral amputees

Authors: *C. M. CIRSTEA[1], H. PENG[2], S. H. FREY[2];
[1]Psychological Sci. and Physical Med. & Rehabil., [2]Psychological Sci., Univ. of Missouri, Columbia, MO

Abstract: Background: Deafferentiation and reduced efferent activity following limb loss precipitate functional reorganization in the sensory and motor cortical maps contralateral to the amputation. Animal models indicate several stages of central eorganization. Changes immediately post-injury involve disinhibition of latent intracortical connections and are followed by synaptogenesis, axogenesis, and a reduction in the number of GABA neurons or its synthetic enzyme glutamic acid decarboxylase. Whether similar mechanisms underlie the post-amputation reorganization in humans is unknown. We addressed this issue through use of noninvasive proton magnetic resonance spectroscopy (1H-MRS). Relative to the hemisphere ipsilateral to the amputation, we predicted that amputees would show evidence of compromised neuronal metabolism (lower N-acetylaspartate, NAA) and reduced inhibition (higher glutamate-glutamine complex, Glx). Methods: Eight (3 female) right-hand dominant, adult (46.5±9.9 years), chronic (12.2±11.4 years), traumatic unilateral hand amputees (one missing the left hand) underwent functional MRI, 1H-MRS (water suppressed Point RESolved Spectroscopy) and structural MRI evaluations. Brain-tissue-corrected absolute concentrations of NAA and Glx were calculated in the cortical sensorimotor hand representations defined functionally: during an fMRI localizer,

amputees executed finger flexion-extension with the intact hand while imagining comparable movements of the absent hand. Results: Significantly lower concentrations of NAA (p=0.005) were found in the former hand territory compared to the intact hand territory. We found a non-significant (p=0.3) trend toward higher Glx concentrations in the contralateral hemisphere. A significant negative correlation was found between contralateral Glx concentrations and time since amputation (r=-0.73). Discussion: Our results provide initial evidence for lower NAA in the former sensorimotor hand territory following unilateral amputation adults. The failure of the trend toward higher Glx to achieve significance may be attributable to the fact that use of the intact hand increases activity within the former hand territory. This may partially offset reductions in intracortical inhibition. The decrease in Glx with increasing time post-injury implies that the balance between excitation and inhibition changes in favor of increases inhibition with experience. Additional work is underway to decipher the functional implications of such change.

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Poster

705. Peripheral and Central Nerve Injury

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Program#/Poster#: 705.03/U9

Topic: C.10. Trauma

Title: Monocarboxylate transporter 1 (MCT1) in perineurial cells is critical for regeneration of peripheral nerves

Authors: *B. M. MORRISON[1], A. TSINGALIA[2], S. VIDENSKY[2], S. LENGACHER[3], L. PELLERIN[4], P. J. MAGISTRETTI[3], T. J. PULLEN[5], G. A. RUTTER[5], J. D. ROTHSTEIN[2]; [1]Neurol., [2]Johns Hopkins Univ., Baltimore, MD; [3]Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland; [4]Univ. of Lausanne, Lausanne, Switzerland; [5]Imperial Col., London, United Kingdom

Abstract: Nerves, particularly regenerating nerves, have high metabolic demands in order to maintain critical axonal functions such as axon transport and membrane depolarization, and Schwann cell functions of myelin clearance, proliferation, and remyelination. We hypothesize that metabolic energy to axons and Schwann cells is supplied locally by lactate, which requires MCTs for transport across membranes. In the peripheral nerve, MCT1 is primarily localized to perineurial cells. Perineurial cells are known to function in maintaining the blood-nerve barrier,

but over the last several years, several studies have expanded our understanding of their role to include early and critical contributions to developing, and possibly regenerating, peripheral nerves. We found that nerve regeneration following a proximal sciatic nerve crush in MCT1 heterozygous null mice (MCT1 Het), which express 50% MCT1 compared to wild-type mice, is markedly delayed as measured electrophysiologically through compound muscle action potentials (CMAPs) or histologically by regenerating nerve counts, neuromuscular junction denervation and muscle atrophy. Though the mechanism for failed axon regeneration is not clear, early increased numbers of myelin degenerating profiles and later reduced percentage of myelinating axons in the MCT1 Het mice suggests that perineurial MCT1 is important for Schwann cell function. We are currently investigating this further using both MCT1 overexpressing lentiviral vector constructs and a recently published MCT1 overexpressing mouse. Through these techniques, we are investigating the capacity of MCT1 upregulation in perineurial cells to restore nerve regeneration in the MCT1 Het mice as well as to accelerate nerve regeneration in MCT1 overexpressing mice. These experiments add to the growing literature on the importance of perineurial cells in nerve regeneration and may provide proof of principle studies for the development of novel therapeutics to accelerate nerve regeneration focused on MCT1.

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Poster

705. Peripheral and Central Nerve Injury

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Jytte og Kai Dahlboms Foundation, DK

Title: Increased membrane inward rectification current in regenerated peripheral motor axons of man and mouse

Authors: M. MOLDOVAN[1,2], S. ALVAREZ[1], *C. KRARUP[2,1];
[1]Univ. of Copenhagen, Copenhagen, Denmark; [2]Rigshospitalet, Copenhagen, Denmark

Abstract: In contrast to the good recovery of conduction, the membrane function of regenerated axons remains persistently abnormal. We investigated the electrophysiological recovery of 3 adult male patients with surgically repaired complete injuries of peripheral nerves of the arm in which the lesions occurred at least 20 cm proximal to the wrist: 2 median nerves at 19 and 26 years after the lesion and 1 ulnar nerve at 22 months after the lesion. The nerves were stimulated at wrist and recordings were carried out from the APB muscle (median) and ADQ muscle (ulnar). The corresponding contralateral nerve was used as control. In all subjects, there was a good recovery of CMAP amplitude ensuring a reliable multiple nerve excitability testing using the TROND "threshold-tracking" protocol. In keeping with our previous results, regenerated axons showed excitability deviations consistent with resting membrane hyperpolarization. The larger threshold increase during hyperpolarizing threshold electrotonus was largely attenuated by 200 ms, when a clear increase in the hyperpolarizing I/V slope could be noted. The 'Bostock' human myelinated axon model indicated that this increase in the inward rectification current (Ih) could not simply be explained by a hyperpolarizing change in membrane potential. More likely, it was attributable to a directly increased inward rectifier conductance (GH). Furthermore, the increase in GH was most prominent in the nerve with shortest time of regeneration. We further investigated these changes during regeneration of tibial nerve following sciatic nerve crush carried out in 2-month C57Bl mice. Robust reinnervation of the plantar muscles occurred about 1 months after the lesion, so that the plantar muscle CMAPs could be tracked. Similar to human regenerated nerves, the largest magnitude deviation in mice was the larger threshold increase during hyperpolarizing threshold electrotonus. Nevertheless, the maximum deviation was reached at about 50 ms and then recovered to about half already by 100 ms. By 5 months of regeneration the abnormal deviations to hyperpolarizing threshold electrotonus showed a partial recovery, although the overall excitability remained markedly reduced. By optimizing the 'Bostock' model to the mouse tibial nerve, we found that in mice, regenerated axons showed a marked increase in GH at 1 month which recovered by 5 months. Taken together, our data suggest that in regenerating axons there is an increased Ih reflecting an increased GH. As GH is mediated by the hyperpolarization-activated cyclic nucleotide-gated channels, it is possible that the increased GH reflects an increased cAMP signaling in regenerating nerves.

Disclosures: M. Moldovan: None. **S. Alvarez:** None. **C. Krarup:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.05/U11

Topic: C.10. Trauma

Support: CAPES

FAPERJ

CNPq

Ministério da Saúde

Title: A combination of schwann-cell grafts, biodegradable conduits and aerobic exercise enhances ischiatic nerve regeneration

Authors: C. O. GOULART[1,2], S. JÜRGENSEN[1,4], A. SOUTO[1], J. T. OLIVEIRA[1], S. DE LIMA[1], C. TONDA-TURO[5], S. A. MARQUES[6], F. M. ALMEIDA[2,3], *A. B. MARTINEZ[1,2]; [1]ICB, UFRJ, Rio De Janeiro, Brazil; [2]Dept. de Patologia - Faculdade de Medicina, UFRJ, Rio de Janeiro, Brazil; [3]Pólo Universitário Macaé, UFRJ, Macaé, Brazil; [4]Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; [5]Dept. of Mechanics, Politecnico di Torino, Turin, Italy; [6]Inst. de Biologia - Dept. de Neurobiologia, UFF, Niterói, Brazil

Abstract: Background: Despite the regenerative potential of the peripheral nervous system, severe nerve lesions lead to loss of target-organ innervation, making complete functional recovery a challenge. Few studies have given attention to combining different approaches in order to accelerate the regenerative process. Objective: Test the effectiveness of combining Schwann-cell (SC) transplantation into a biodegradable conduit, with treadmill training (TMT) as a therapeutic strategy to improve the outcome of repair after mouse nerve injury. Methods: Ischiatic nerve transection was performed in adult C57BL/6 mice; the proximal and distal stumps of the nerve were sutured into the conduit. Four groups were analyzed: those with acellular grafts (DMEM), with Schwann cell grafts (3x105/2 µL; SC), with treadmill training (TMT), and with treadmill training and Schwann cell grafts (TMT+SC). Locomotor function was assessed weekly by Ischiatic Function Index (SFI) and Global Mobility Test (GMT). Animals were anesthetized after eight weeks, and the ischiatic nerve was dissected for morphological analysis. Results: The combined therapies improved nerve regeneration, and increased the number of myelinated fibers and myelin area compared to the DMEM group. Motor recovery was accelerated in the TMT+SC group, which showed significantly better values in SFI and in GMT than in the other groups. The TMT+SC group showed increased levels of trophic-factor expression compared to DMEM, contributing to the better functional outcome observed in the former group. Conclusion: These data provide evidence that this combination of therapeutic strategies can significantly improve functional and morphological recovery after ischiatic injury.

Disclosures: **C.O. Goulart:** None. **S. Jürgensen:** None. **A. Souto:** None. **J.T. Oliveira:** None. **S. de Lima:** None. **C. Tonda-Turo:** None. **S.A. Marques:** None. **A.B. Martinez:** None. **F.M. Almeida:** None.

Poster

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Support: NIH-NINDS (1R01NS072388).

Title: Role of TNFR family members in Wallerian Degeneration

Authors: ***K. GAMAGE**[1], S. NACHUM[1], R. PARK[1], A. SPANO[1], A. ERISIR[2], C. DEPPMANN[1];
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Abstract: Developing neural circuits undergo a wide range of refinement in the form of axon degeneration. In development, axon degeneration accounts for the clearing of weak or unwanted axons and branches. Axon degeneration is also a hallmark of a variety of pathologies such as neurodegenerative disease (eg. Alzheimer's, Parkinson's, ALS) and nerve injury. Recent studies have revealed that members of the Tumor Necrosis Factor Receptor (TNFR) superfamily are required for developmental degeneration in Peripheral Nervous System (PNS) and Central Nervous System (CNS). In this study we sought to determine whether these TNFR family members are also involved in injury-induced degeneration. Using an *in vitro* microfluidic injury paradigm we assessed the neurons lacking various TNFR family members. We found that inhibition of these receptor or their downstream signaling pathways delay Wallerian degeneration. We will also discuss our recent *in vivo* findings, which employ electron microscopy to study the degeneration of transected sciatic nerves in different TNFR family member knockout mice.

Disclosures: **K. Gamage:** None. **S. Nachum:** None. **R. Park:** None. **A. Spano:** None. **A. Erisir:** None. **C. Deppmann:** None.

Poster

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Shriners Hospitals for the children

Title: Constitutively active ErbB2 revives chronically denervated, atrophied Schwann cells

Authors: *S. HAN[1], J. ZHAI[1], M. WRIGHT[2], W. THOMPSON[3], T. FERGUSON[1], Y.-J. SON[1]; [1]SHPRC, Temple. Univ. Sch. of Med., Philadelphia, PA; [2]Arcadia Univ., Glenside, PA; [3]Texas A&M Univ., College Station, TX

Abstract: Peripheral nerves regenerate, but rarely over long distances, which limits functional recovery of many patients with proximal nerve injuries. A principal reason for the lack of long-distance regeneration is atrophy of Schwann cells in the distal nerves; they become less supportive with extended denervation time. With the ultimate goal of developing a potent treatment that enables long-distance regeneration, we investigated whether constitutively active ErbB2 (caErbB2) prevents Schwann cell atrophy and/or revives atrophied Schwann cells. For this purpose, we used S100-rtTA;TetO-NeuNT double transgenic mice in which expression of caErbB2 is induced selectively in Schwann cells by a Tet-On system. Following complete transection of sciatic nerve, Schwann cells in the distal nerves were kept denervated for 2 months by blocking axon regeneration from proximal nerves. The transgenic mice were then fed with regular (control group) or doxycycline -containing food (experimental group) for 2 weeks before histological and ultrastructural analyses. The distal nerves treated with doxycycline showed several notable features: 1) they were much thicker than the severely atrophied nerves of control animals; 2) they were filled with many more Schwann cells and processes; 3) most Schwann cells were large and wrapped by basal lamina, unlike most of those in controls, which were extremely atrophied and had lost their basal lamina sheath; 4) there were fewer macrophages than in controls, another indication that the Schwann cells expressing caErbB2 were being revived. Intriguingly, several growth-associated factors such as c-Jun were highly upregulated in the dox-treated distal nerves. Our results thus far are promising and suggest that ErbB2 can reverse Schwann cell atrophy and may promote long-distance regeneration of injured peripheral nerves.

Disclosures: S. Han: None. **J. Zhai:** None. **T. Ferguson:** None. **Y. Son:** None. **M. Wright:** None. **W. Thompson:** None.

Poster

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Support: Grant-in-Aid for Scientific Research (C) from the JSPS

Title: An EGFR inhibitor induces Schwann cell proliferation and promotes functional recovery with remyelination in injured peroneal nerve after end-to-side neurorrhaphy

Authors: *M. ENOMOTO[1], M. UKEGAWA[1], H. KABURAGI[1], T. HIRAI[1], A. OKAWA[1], K. YAGISHITA[2], Y. WAKABAYASHI[1];
[1]Orthopaedic & Spinal surgery, [2]Hyperbaric Med. Ctr., Tokyo Med. & Dent. Univ., Tokyo, Japan

Abstract: It is known that the epidermal growth factor receptor (EGFR) inhibitors are anti-cancer agents with potential results in cell cycle arrest, apoptosis or dedifferentiation of cancer cells. In the case of nerve regeneration, local administration of EGFR inhibitors promotes significant regeneration of injured optic nerve fibers (Koprovica et al. Science 2005). However, it is not clear whether EGFR inhibitors would promote peripheral nerve regeneration after injury. In this study, we tested whether the inhibition of EGFR regulates neurite extension in dorsal root ganglia (DRG) neurons or Schwann cell proliferation *in vitro* and nerve regeneration in injured proneal nerves after end-to-side neurorrhaphy. The *in vitro* results showed that regeneration of neurites from explanted DRG neurons was not significantly accelerated by administration of EGFR inhibitor AG1478. In contrast, the number of Schwann cells was increased with AG1478 treatment as shown by a viability assay. Rat peroneal nerve was cut and then end-to-side neurorrhaphy, coaptation of the distal stump of a transected peroneal nerve to the tibial nerve, was performed 3 weeks later. At the same time, AG1478 (10 nM) was infused by osmotic pump at the injury site for 7 days. After the surgery, motor score was increased significantly at 2 weeks and the amount of myelin in the peroneal nerve was increased significantly at 4 weeks in the AG1478 infusion group. These findings suggest that local administration of EGFR inhibitor AG1478 has the potential to increase functional recovery after peripheral nerve injury.

Disclosures: **M. Enomoto:** None. **M. Ukegawa:** None. **H. Kaburagi:** None. **T. Hirai:** None. **A. Okawa:** None. **K. Yagishita:** None. **Y. Wakabayashi:** None.

Poster

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Topic: C.10. Trauma

Support: Fapesp

CNPq

Title: Tempol antioxidant rescues spinal motoneurons from degeneration and prevent apoptotic events after peripheral axotomy in neonatal rats

Authors: ***G. CHIAROTTO**, L. DRUMOND, G. CAVARRETTO, A. BOMBEIRO, A. L. R. OLIVEIRA;
Univ. of Campinas, Campinas, Brazil

Abstract: Neonatal spinal cord motoneurons and primary sensory neurons are susceptible to degeneration after peripheral axotomy. In rats, sciatic nerve transection on the day of birth leads to degeneration of 53% to 89% of axotomized sensory neurons and 80% of spinal motoneurons. Such neuronal loss is concurrent with a prominent loss of synapses, seen by synaptophysin immunolabeling, decreasing complexity of spinal circuits. Since most of the neuronal loss is due to oxidative stress and apoptotic mechanisms, drugs that act as reactive oxygen species (ROS) chelators contribute to neuroprotection and, in turn, can be of clinical interest. Among the neuroprotective substances, neurotrophins are key molecules. Nevertheless, tempol (4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl), which is considered a nitroxide or a free radical that undergoes one or two reduction cycles, may represent a new alternative to injuries that lead to apoptotic death of neurons and glial cells. In this sense, we investigated the neuroprotective effects of tempol in different concentrations after sciatic nerve transection in neonatal rats. For this, two days old pups underwent sciatic nerve axotomy and were divided in vehicle group (saline) and tempol treated group. Intraperitoneal injections of tempol at the concentrations of 12, 24 and 48 mg/kg were performed 10min, 6h, and every 24h up to 1 week after injury. The animals were euthanized after the predetermined survival time points post lesion and the lumbar intumescence was dissected out. Cross sections (12 μm) were used for Nissl staining to evaluate motoneuron survival, Tunel technique to detect occurrence of apoptotic nuclei and synaptophysin immunolabeling (24 hours after lesion). qRT-PCR was used to investigate the expression of pro and anti-apoptotic molecules Caspase 3, Bax and Bcl2, 12 and 24 hours after

lesion (24mg/kg). The results demonstrated that tempol increased neuronal survival in all survival times studied and all concentrations investigated when compared with the vehicle groups. However, the concentration of 24mg/kg showed better results when compared with 12mg/kg and 48mg/kg. The TUNEL-positive cell number decreased in tempol-treated animals. qRT-PCR results indicated differential increase in Caspase 3 (3-fold), Bax (13-fold) and Bcl2 (28-fold) gene expression, after 12h following axotomy and tempol treatment. In conclusion, tempol administration has proven to be neuroprotective after neonatal nerve injury, leading to improved motoneuron survival, synapse preservation and minimizing apoptosis.

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Poster

705. Peripheral and Central Nerve Injury

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Topic: C.10. Trauma

Support: Adelson Medical Research Foundation

Title: Aged Schwann cell grafts impair regeneration of young neurons

Authors: *J. SCHEIB[1], A. R. JUMAN[1], A. HOKE[2];
[2]Neurol., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Increasing age has a negative impact on nerve regeneration. Multiple explanations for this have been proposed, including: 1) older neurons may be less able to regenerate, 2) older Schwann cells may be less supportive of regeneration, and 3) there is a delay in degeneration and clearance of axonal and myelin debris. To test these hypotheses, I performed nerve grafting with 2 month old and 18 month old Brown-Norway male rats. I sutured 1 cm sciatic nerve grafts from young or aged rats into sciatic nerves of young or aged rats. Regeneration was allowed to occur until the tissues were harvested at 3 days, 7 days, 2 weeks, and 6 weeks. Once harvested, the grafts and distal stumps were examined for debris clearance and axonal regeneration. My data suggest that placement of aged nerve grafts into nerves of young rats impairs regeneration of young neurons, while the placement of young nerve grafts into the nerves of aged rats improves regeneration of aged neurons. Moreover, the clearance of myelin debris in young grafts is slowed when sutured into the nerves of aged rats, and clearance in aged grafts is faster when sutured into

young rats. This data is in agreement with previous studies that suggest delayed clearance inhibits regeneration in aged animals. We are currently using this grafting model to explore the causes of slow clearance, whether it be delayed Wallerian degeneration, cytokine secretion, macrophage infiltration, and/or phagocytosis by Schwann cells and macrophages.

Disclosures: J. Scheib: None. **A.R. Juman:** None. **A. Hoke:** None.

Poster

705. Peripheral and Central Nerve Injury

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Topic: C.10. Trauma

Support: Craig H. Neilsen Foundation Postdoctoral Fellowship

Generous private donations

Title: Conditional co-deletion of PTEN and SOCS3 accelerates functional recovery following peripheral nerve damage

Authors: *Z. R. GALLAHER, O. STEWARD;
Anat. & Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: Following damage to the nervous system, regeneration is necessary to restore lost connections. Unfortunately, regeneration is extremely poor in the adult central nervous system. Recent work has shown that conditional genetic deletion of phosphatase and tensin homolog (PTEN) promotes regeneration within the optic nerve and cortical spinal tract by releasing inhibition on the PI3K pathway. Furthermore, co-deletion of PTEN and suppressor of cytokine signaling 3 (SOCS3), a negative feedback regulator of the Jak/Stat pathway, has an additive effect on regeneration in the optic nerve. It has not been shown if a similar strategy can enhance regeneration in the peripheral nervous system, an area where regeneration is possible but rarely complete. Here we tested the hypotheses that (1) deletion of PTEN in the dorsal root ganglia (DRG) would improve the recovery of sensory functions following sciatic nerve crush and (2) that PTEN and SOCS3 co-deletion would have an additive effect on recovery. To determine the effects of injury on PI3K and Jak/Stat signaling, we performed a mid-thigh level sciatic nerve crush on adult male mice and extracted the fourth and fifth lumbar DRG for immunofluorescent and Western blot analysis at 1, 3, 7, 15, and 30 days post-injury. In control DRG, PTEN was

expressed in small diameter neurons. Conversely, phosphorylation of ribosomal protein S6, a downstream target of PI3K signaling, was active in large diameter neurons. Sciatic nerve crush increased p-S6 expression with a peak at 3 days post-injury. Similarly, p-Stat3 expression was drastically increased 1 and 3 days post-injury before returning to baseline levels after an increase in SOCS3 expression. To determine the effects of PTEN and PTEN/SOCS3 co-deletion on functional recovery, the fourth and fifth lumbar DRG of adult male PTEN f/f and PTEN/SOCS3 f/f mice were injected with AAV-Cre two weeks prior to sciatic nerve crush. Noxious heat and pressure sensation were measured weekly using a Hargreaves apparatus and von Frey filaments, respectively. Neither PTEN deletion nor PTEN/SOCS3 co-deletion had an effect on the final recovery plateaus of noxious heat or pressure sensation; however, PTEN/SOCS3 co-deletion accelerated the recovery of both sensory measures. Specifically, hind paw withdrawal latency to heat and withdrawal threshold to pressure were significantly lower in PTEN/SOCS3 deleted mice at 14 and 21 days post-injury, respectively. These results suggest that inhibition of PTEN and SOCS3 can accelerate regeneration. This may be especially important when distances from injury site to peripheral target are longer, as is often the case for human patients suffering from nerve damage.

Disclosures: Z.R. Gallaher: None. **O. Steward:** None.

Poster

705. Peripheral and Central Nerve Injury

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Topic: C.10. Trauma

Title: Quantitative EEG (qEEG) as a biomarker for traumatic brain injury (TBI) in absence of signs on MRI imaging

Authors: *G. NEWMAN, W. E. KOZACHUK, S. HETHER;
The Neurosci. Team, Lutherville, MD

Abstract: b>Objective: To assess the efficacy of use of quantitative electroencephalography (qEEG) as a biomarker for TBI, abnormal brain patterns in electro-cortical activity were correlated with neurological symptoms and neuropsychological testing in five patients who developed moderate symptoms after a specific, localized TBI. **Methods:** The results of neuropsychological testing and patients' subjective descriptions of symptoms were correlated in five cases with qEEG measures of absolute power and coherence, using Brain Master and

Deymed 24 channel hardware, applied with full-head caps, with Neuroguide database for analysis. These results were also compared with MRI results. Average split half and test-retest reliability measures were all at .95 and above. **Results**: qEEG was able to map the predicted brain deficits in TBI to the specific quadrant of the brain, in the presence of normal brain MRI scans. qEEG brain mapping abnormalities correlated with the predicted quadrant from abnormalities noted on neuropsychological exam, and to quadrant predicted by checklist of symptoms endorsed by the patients and neurological examination. The main qEEG abnormalities observed within the identified quadrant were elevated beta and delta measures, and elevated or deficient coherence. **Conclusions**: Early utilization of qEEG is a reliable and valid biomarker for TBI in patients who develop mild to moderate symptoms. It is suggested that early application of this modality (e.g. in E.R. or Shock-Trauma) could be used to correlate with other imaging modalities and guide administration of medical therapy. Word Count: 261

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Poster

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Support: Medical Research Council (Medicine)

Lund University

Region Skåne

Title: Expression of Heat Shock Protein 27 (HSP 27) and axonal outgrowth after nerve injury and repair

Authors: *L. B. DAHLIN[1,2], J. ENANDER[2], B. HAZER[2], C. LINDWALL-BLOM[3], L. STENBERG[2];
[1]Scania Hand Ctr. AB, S-200 42 Malmo, Sweden; [2]Dept Clin. Sci. Malmö - Hand Surgery, Lund Univ., Malmö, Sweden; [3]Cellectricon AB, Mölndal, Sweden

Abstract: Heat shock protein 27 (HSP 27) is considered to be neuroprotective and is increased after a nerve crush injury; i.e. a clinically less relevant nerve injury. Our aim was to analyse expression of HSP 27 after nerve injury and repair. Rat sciatic nerves were transected and

repaired with sutures either directly or after a one-week delay. After one (direct repair) or two weeks (direct and delayed repair) the nerves were harvested, longitudinally cryosectioned and stained for neurofilaments and HSP 27. The distance of axonal outgrowth, detected by neurofilament staining, and the intensity of HSP 27 staining were measured at the site of lesion (SNL) and in the distal nerve segment (SND) in each nerve. Neurofilaments were present in all examined nerves with, as expected, reduced distance of axonal outgrowth in the repaired nerves that were evaluated after one week. All sections were positively labelled for HSP 27 with immunoreactivity in both axons and in Schwann cell columns at SNL and SND with a significantly lower staining intensity at both locations in repaired nerves evaluated after one week. The outgrowth distance correlated with HSP 27 labelling intensity both at SNL and at SND. There was no difference in distance of axonal outgrowth or intensity of HSP 27 between nerves repaired directly or after a one week delay; i.e. both evaluated two weeks after injury, indicating that a delayed repair improves axonal outgrowth, but a delay does not affect expression of HSP 27. We conclude that there is a gradual increase in expression of HSP 27 after nerve injury and repair, which correlates with axonal outgrowth.

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Poster

705. Peripheral and Central Nerve Injury

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Topic: C.10. Trauma

Support: Texas Medical Research Collaborative

Title: Radio-frequency fields elicit neuroma pain

Authors: ***R. GRANJA-VAZQUEZ**[1,2], B. R. JOHNSTON[1,2], M. I. ROMERO-ORTEGA[1,2]; [1]Bioengineering, Univ. of Texas At Arlington, Arlington, TX; [2]Bioengineering, UTSW Med. Ctr., Dallas, TX

Abstract: Following peripheral nerve amputation, a high percentage of patients develop bulbous neuromas, which cause chronic pain elicited by mechanical and/or thermal stimuli. Anecdotal evidence suggests that radio-frequency (RF) stimulus can also elicit pain in amputation neuromas. In this study, we directly ascertained the effect of RF-stimulus to cause pain in an

animal amputation neuroma model. Sixteen adult female transgenic Wistar Rats (Thy1.2 promoter ChR2-Venus) that express ChR2 primarily in mechanoreceptive fibers underwent a tibial neuroma transposition (TNT) surgery. Four animals with sham surgeries were included as controls. The animals were tested for pain responses weekly for 2 months after surgery by observers blinded to the experimental groups. Four pain modalities were evaluated: a) RF stimulus (915 MHz, 750 mW/m2; Andrew RFID-900-SC antenna; field measured by Tenmars TM-196 Meter), b) Von Frey mechanical stimulus (300 g filament), c) thermal stimulus (6°C skin temperature increase), and d) optogenetic stimulus (20 mW, 473 nm). The pain responses were scored in repeated trials according to the following criteria: 0-no response, 1-paw withdrawal, and 2-paw shaking, stretching, licking or vocalizations. The pain responses were added over 8 trials and analyzed via non-parametric Mann-Whitney test. As expected, TNT-neuroma animals had significantly higher pain responses to the established Von Frey and thermal assays when compared to the non-injured animals ($p<0.05$). In addition, RF stimulus consistently elicited responses that resulted in a cumulative pain score of 2.82 per TNT-neuroma animal per trial (as compared to a score of 0.06 for non-injured animals). However, laser stimulus was insufficient to elicit pain suggesting that mechanoreceptive fibers may not contribute significantly to neuroma pain perception. This study demonstrates that RF fields can induce pain in injured nerves, particularly in those forming neuroma-like growth at the transected end. Further, our results suggest that strategies designed to shield common RF waves at the transected nerve end, may provide pain relief from this type of insult to patients suffering from neuroma pain.

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Poster

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Support: NS083377-01 National Institutes of Health/ National Institutes of Neurological Disease and Stroke

Title: Diffusion tensor tractography study of sensorimotor pathways in unilateral hand amputees

Authors: *H. PENG[1], C. M. CIRSTEA[2], K. F. VALYEAR[1], S. H. FREY[1];
[1]Psychological Sci., [2]Psychological Sci. and Physical Med. & Rehabil., Univ. of Missouri-Columbia, Columbia, MO

Abstract: Background: Injury to the peripheral nerves, such as hand amputation, is associated by large-scale functional reorganization in sensory and motor cortices contralateral to the injury. Animal models indicate structural changes in the gray matter of these cortices as well as in other brain regions, such as thalamus, brainstem and the spinal cord. Evidence for activity-dependent changes in white matter integrity in animal models or humans is less clear. We used diffusion tensor imaging (DTI) to test the hypothesis that the chronic unilateral hand loss is associated with changes in structural integrity in major afferent (medial lemniscus, ML) and efferent (corticospinal tract, CST) pathways of the hemisphere contralateral to the amputation. Methods: We tested 13 (3 female), right hand dominant, chronic (mean+SD=23+14years), traumatic unilateral, hand amputees (4 missing the left hand) and 13 age-, gender- and handedness matched controls. Single-shot spin-echo echo-planar DTI images were acquired (3T Siemens Allegra MRI scanner). Probabilistic tractography was used to delineate the ML and CST in each hemisphere (FMRIB Software Library software). A seed for the ML was defined anatomically at the brainstem level with a thalamic waypoint and cortical terminus. A seed for the CST was defined in the cerebral peduncle with a waypoint in the internal capsule and cortical terminus. Volume, mean diffusivity (MD), and fractional anisotropy (FA) were computed for the tracts of each hemisphere in both groups. Results: We failed to detect any significant differences in tract volume, MD or FA of ML or CST between hemispheres in controls. By contrast, amputees exhibited significant asymmetries in both ML and CST contralateral to the amputation. Relative to the ipsilateral side, contralateral ML exhibited decrease in volume and increase in MD. FA was not significantly different between ipsi- and contralateral ML. Compared to ipsilateral CST, contralateral CST displayed decrease in FA and increase in MD. There were no significant between-hemisphere differences in CST volume. Discussion: Our preliminary results suggested that the structural integrity of contralateral ML and CST is altered following unilateral amputation. Hand loss precipitates a major reduction in afferent signals and this may account for ML changes in MD and volume. Axonal degeneration could be a potential candidate for these changes. An extensive reorganization in the motor system has been also reported after hand loss and this might account for the asymmetry in CST FA and MD. The absence of changes in CST volume is consistent with the preservation of the descending outputs.

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Poster

705. Peripheral and Central Nerve Injury

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Support: NIH KO8 award number 1 K08 AR060164-01A

Title: 4-aminopyridine (4-ap) locally delivered from plga encapsulated particles is functionally potent on peripheral nerve crush injury

Authors: *K.-C. TSENG[1], H. LI[1], M. NOBLE[2], J. ELFAR[1];
[1]Orthopaedics, [2]Biogenetics, Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Severe peripheral nerve crush injury (PNCI) often spares few neurons which may nonetheless recover to support near-normal function. However, even when intact, dysfunction in these fibers precludes functional recovery. Recently, we have found a large effect of continuous systemic 4-Aminopyridine (4-AP) in a model of severe PNCI which injures all fibers where treatment quickly leads to transient recovery and continues to recover quicker than control group. 4-AP is an FDA-approved potassium-channel blocker used in multiple sclerosis to elongate the duration of neuronal action potentials. We reasoned that the treatment of PNCI in this model involves stabilization of injured but not severed axons by stabilizing demyelinated areas. We hypothesized that local delivery and sustained release of 4-AP can continuously prolong action potentials on injured nerve and may promote remyelination through improved conduction. We formulated slow release particles and films of Poly (lactic-co-glycolic acid) (PLGA) able to release 4-AP slowly over time to prolong the treatment effect and test our hypothesis. Two fabrication methods (emulsion and solvent casting) were employed to encapsulate 4-AP into carriers with distinct geometries capable of different drug loading capacities, release rates, and degradation rates. Crush injuries were performed in mice and (4-AP)-PLGA carriers were implanted directly at the site of the injury on sciatic nerve. Large motor functional improvements were evident in both (4-AP)-PLGA carriers transplanted groups over untreated controls as measured using the Sciatic Function Index and nerve conduction velocity. Immunohistochemical analyses, revealed ultrastructral improvements including more myelin three-weeks post-treatment in addition to increased expression of neurotrophic factors - known to promote neuronal myelin production in-vitro. Taken together, we believe mice treated with 4-AP-PLGA slow release formulations reveal a translational treatment for PNCI with relevance to human injury.

Disclosures: K. Tseng: None. **H. Li:** None. **M. Noble:** None. **J. Elfar:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.17/U23

Topic: C.10. Trauma

Support: NIH Grant NS057190

NIH Grant HD032571

Title: Optical stimulation promotes motoneuron-derived sex steroid synthesis

Authors: *P. J. WARD, A. W. ENGLISH;
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Abstract: Moderate daily exercise promotes the regeneration of axons in cut peripheral nerves. Androgen receptor signaling is required for activity-enhanced (exercise and electrical stimulation) axon regeneration in both sexes. The source of the ligands for the androgen receptor, androgens, is not known. We hypothesized that axotomized neurons are capable of modulating sex steroid synthesis and that increased neuronal activity may enhance these pathways to facilitate axon regeneration, specifically toward androgen pathways. Here, we investigated the effects of peripheral nerve transection and optical activation (as a model of activity) on the neuronal expression of 5 alpha-reductase (5αR), which converts testosterone into dihydrotestosterone, and 17 beta hydroxysteroid dehydrogenase (17βHSD), which converts steroid precursors into testosterone. First, we used male and female mice that express YFP under the control of the thy-1 promoter, which restricts YFP to neurons, and in the spinal cord, mainly motoneurons. We combined cell gradient centrifugation, fluorescence activated cell sorting, and qPCR to determine the mRNA expression in YFP+ neurons of the lumbar spinal cord of intact mice and mice in which the sciatic nerve was transected. One day (24 hours) after axotomy an 8.6 fold increase in 5αR was found in males compared to intact controls. A 1.7 fold increase was found in females. At the same time, a 1.2 fold increase in 17βHSD was found in both males and females. Next, we used male mice that express ChR2-YFP under the control of the ChAT promotor, which restricts ChR2-YFP to cholinergic neurons, and in the spinal cord, only motoneurons. One group of mice was optically stimulated for one hour, and the sciatic nerve was transected. The other groups were transection only and intact ChAT-ChR2-YFP mice. The transected only group had a similar increase in 5αR, 9.5 fold compared to intact motoneurons 24 hours after transection. However, the optically stimulated group had a 243 fold increase in 5αR compared to intact motoneurons 24 hours after transection. Furthermore, activity (in the form of motoneuron specific optical activation) dramatically increased the mRNA of 5αR. This result supports our hypothesis that activity enhances androgen receptor signaling in order to enhance

axon regeneration. The time course of this expression, and the effects of activity based therapies on these enzymes will be important considerations if activity based therapies are to be effectively applied to the diverse population of nerve injury patients.

Disclosures: P.J. Ward: None. **A.W. English:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.18/U24

Topic: C.10. Trauma

Title: Neuroanatomical characterization of reactive gliosis in the brainstem after hypoglossal nerve injury using a newly developed whole-mount imaging technique

Authors: *P. KHALILI[1], A. FROSTELL[2], S. THAMS[2], B. MEIJER[2], M. SVENSSON[2]; [1]Karolinska Institutet/Universitetssjukhuset, Stockholm, Sweden; [2]Karolinska Institutet, Stockholm, Sweden

Abstract: Peripheral nerve injury induces an array of retrograde responses, including cell death, tissue remodelling and reactive gliosis. The latter is a key event, which has been implicated in as well protective as harmful processes. The two main actors in the glial response are astrocytes and microglia, and their activational patterns are believed to be in part dependent on each other. Even though reactive gliosis has been studied from many different aspects, certain key features, such as organisation, migration and relation to neurons and vasculature remain poorly understood on the intermediate anatomical level. In order to study these processes, our laboratory therefore recently constructed a light sheeth fluorescence microscopy (LSFM)-based imaging platform, allowing for imaging of large specimens. By combining this new imaging technique with methods for optical tissue clearing, such as CLARITY, we are now able to image large specimens and whole-mount preparations at high acquisition speeds with a resolution near the diffraction limit. Sprague Dawley rats were subjected to either a hypoglossal nerve avulsion, transection, crush injury or sham operation. Rats were sacrificed at 14 and 28 days postoperatively; then perfused, sectioned in thick sections (500-1000 μm) or maintained as whole-mount specimens. The tissue was processed according to the CLARITY-protocol and then stained for glial fibrillary acidic protein (GFAP), Iba1 and Tuj-1. Images were acquired using our imaging platform and 3D reconstructions were generated after further processing. We are now in the process of defining qualitative and quantitative parameters to be used for evaluation of the

reactive gliosis on the intermediate neuroanatomical scale. Using this whole-mount imaging approach, we aim to obtain a more holistic perspective of how glial cells react to injury and how these cells interact with different cell types in their surroundings.

Disclosures: **P. Khalili:** None. **A. Frostell:** None. **S. Thams:** None. **B. Meijer:** None. **M. Svensson:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.19/U25

Topic: C.10. Trauma

Support: Spinal Cord Injury Sucks Foundation

Unite 2 Fight Paralysis

Title: High content screening of oncogenic and tumor suppressive transcription factors identifies novel promoters of neurite outgrowth

Authors: ***M. SIMPSON**, D. COLEY, Z. WANG, M. BLACKMORE;
Marquette Univ., Milwaukee, WI

Abstract: The intrinsic ability of neurons to extend axons is an important determinant of recovery from injury to the central nervous system. Because axon growth demands significant production of cellular material, it has been proposed that genes that are known to regulate cellular growth and division in dividing cells may serve to regulate axon growth ability in post-mitotic neurons. To explore this idea systematically we used publicly available datasets to assemble a list of genes implicated in cellular growth as either oncogenic or tumor suppressive. We then focused on the 209 transcription factors represented in this list, reasoning that transcriptional regulation is well positioned to govern the overall growth state. Of the 209 transcription factors, the Allen Brain Atlas indicated that 137 are present in adult and/or embryonic murine cerebral cortex. To this set we added an additional 51 cortically expressed genes that are close family members to the original 209 transcription factors. We obtained a library of human transcription factors, and using a high content screening approach with cultured cortical neurons tested the effect of 47 transcription factors of interest on axon length. This screen identified seven transcription factors that affected neurite outgrowth, two positively and

five negatively, representing a hit rate of nearly 15%. At least two of these factors have not previously been studied in the nervous system or linked to axon growth, yet western blotting and immunohistochemistry have confirmed endogenous neuronal expression. In addition we have tested VP16 and Engrailed chimeras that act as transcriptionally active and repressive mutant forms, respectively. Intriguingly, we have found examples in which transcriptional repressors that inhibit neurite growth are converted to growth promoters by addition of the VP16 motif. Overall, the high hit rate in this preliminary screen supports the hypothesis of conserved growth mechanisms in neuronal and non-neuronal systems alike, and suggests a promising direction in the ongoing search for additional pro-regenerative genes.

Disclosures: **M. Simpson:** None. **D. Coley:** None. **Z. Wang:** None. **M. Blackmore:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.20/U26

Topic: C.10. Trauma

Support: Svenska Läkaresällskapet, SLS-251901

Svenska Läkaresällskapet, SLS-97641

Title: Gene expression in ventral root replantation

Authors: ***M. K. SKOLD**[1,2], M. GÜNTHER[1];
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Abstract: Replantation of avulsed spinal ventral roots has been show to enable significant and useful regrowth of motor axons in both experimental animals and in human clinical cases, making up an interesting exception to the rule of unsuccessful neuronal regeneration in central nervous system. Compared to avulsion without repair, ventral root replantation seems to rescue lesioned motoneurons from death. In an atempt to map the temporal genetic activity patterns at different time points after avulsion and replantation we have analyzed gene expression in response to both acute ventral root avulsion and replantation as well as after avulsion with delayed replantation. Microarray analysis of ventral spinal cord 24h after acute avulsion and replantation showed regulation of genes related to neurotransmission not seen after avulsion

alone while avulsion only lead to regulation of genes related to inflammation not seen after replantation. When replantation was delayed 24h after avulsion regulation of genes related to neurotransmission was still higher compared to control but after 48h this activity was significantly declined. On the other hand could genes related to inflammation be seen at a higher level at replantation 24 and 48h after avulsion compared to acute replantation and apoptotic regulation was increased in replantation at 24 and 48h compared to acute avulsion/replantation. We conclude that it is possible to do a detailed analysis of the temporal genetic activity profiles after avulsion and replantation of spinal ventral roots. In our study significant differences in genetic activity can be detected between the different timepoints of replantation of spinal ventral roots and controls and our data indicate that the axonal regenerative response from replantation is initiated at an earlier stage than the possible differences in terms of neuron survival. Hopefully studies like this can be useful in finding and biologically defining the time point for replantation after avulsion of spinal ventral roots optimal for nerve regeneration.

Disclosures: **M.K. Skold:** None. **M. Günther:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.21/U27

Topic: C.10. Trauma

Support: Adelson Medical Research Foundation

Title: Defining the astrocyte CSPG and ECM transcriptome *in vivo* by cell-type specific analysis of actively translated mRNA

Authors: ***J. E. BURDA**[1], Y. AO[1], F. GAO[2], G. COPPOLA[3,4,5], M. V. SOFRONIEW[1]; [1]Neurobio., [2]Program in Neurogenetics, [3]Psychiatry, [4]Neurol., [5]Semel Inst. for Neurosci. and Human Behavior, UCLA, Los Angeles, CA

Abstract: Chondroitin sulfate proteoglycans (CSPGs) are critical components of the central nervous system (CNS) extracellular matrix (ECM). CSPGs show significant elevations at sites of neural injury, where they are implicated in the restriction of nerve regeneration and plasticity. Based largely on *in vitro* data, reactive astrocytes are often regarded as the main source of CSPG upregulation following traumatic spinal cord injury (SCI) and other CNS insults. However, various other cell types in the complex multi-cellular response generated by CNS injury also

have the capacity to produce CSPGs and other ECM molecules. Methods of quantitative gene expression have, until recently, not been able to discriminate which cell types are responsible for production of specific molecules *in vivo*. To begin investigating the relative contribution of astrocytes to the production of CSPGs and other ECM components after SCI, we are using the transgenically targeted (*J Neurosci 28:7231; 2008*) RiboTag procedure (*PNAS 106:13939; 2009*) to determine astrocyte selective transcriptome profiles *in vivo*. Using RNA-Seq, we are examining CSPG-related gene expression from astrocyte-specific ribosome-associated mRNA isolated from mouse spinal cord after SCI. Bioinformatics analyses reveal CSPG and ECM fingerprints unique to injury-reactive astrocytes. Reactive astrocyte CSPG- and ECM-related expression patterns differ significantly from whole injured tissue mRNA as well as from astrocyte-mRNA-depleted whole injured tissue mRNA. These findings provide qualitative and quantitative measurement of the extent to which astrocytes contribute CSPGs and other ECM components to traumatic CNS lesions and uninjured tissue. Further investigation into the relative extent, and timing, of CSPG and ECM production specifically by astrocytes, will aid our evolving understanding of mechanisms influencing post-injury neuroregeneration and repair.
Funded by the Adelson Medical Research Foundation.

Disclosures: J.E. Burda: None. **Y. Ao:** None. **F. Gao:** None. **G. Coppola:** None. **M.V. Sofroniew:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.22/U28

Topic: C.10. Trauma

Support: Deutsche Forschungsgemeinschaft (DFG)

Title: Transcriptome network analysis identifies a developmental switch gene that limits regenerative ability in the adult CNS

Authors: *A. TEDESCHI[1], S. DUPRAZ[1], C. LASKOWSKI[1], J. XUE[2], T. ULAS[2], M. BEYER[2], J. L. SCHULTZE[2], F. BRADKE[1];
[1]German Ctr. For Neurodegenerative Dis. DZNE, Bonn, Germany; [2]Genomics and Immunoregulation, LIMES-Institute, Univ. of Bonn, Bonn, Germany

Abstract: Traumatic central nervous system (CNS) injuries often result in permanent disabilities due to axon regeneration failure. Mechanistically, both non-permissive environment and reduced intrinsic growth ability have been proposed to account for the regenerative failure in the adult. While progress has been made in characterizing extracellular growth inhibitors expressed in the adult CNS, our current understanding of the mechanisms that lead to neurons losing their ability to regenerate is still fragmentary. Here we used a systematic and unbiased approach to identify genes whose expression correlated both positively with loss of axon elongation during later stages of embryonic development, and negatively with conditions necessary to gain growth competence in the adult. RNA sequencing (RNA-Seq) screening identified a developmental switch gene that limits axon growth and regeneration. *In vitro* and *in vivo* silencing or pharmacological blockade following administration of an important therapeutic class of drugs promoted axon growth and CNS regeneration. Given that our pharmacological approach is already used clinically to manage a wide range of neurological disorders, our results could significantly impact on the development of therapies aimed at promoting plasticity-related structural changes and regeneration following a variety of CNS trauma.

Disclosures: **A. Tedeschi:** None. **S. Dupraz:** None. **C. Laskowski:** None. **J. Xue:** None. **T. Ulas:** None. **M. Beyer:** None. **J.L. Schultze:** None. **F. Bradke:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.23/U29

Topic: C.10. Trauma

Support: R24HD050846

Title: Multiplexed digital counting of mRNA transcripts representing immune/inflammation pathways over time after experimental spinal cord trauma

Authors: *S. M. KNOBLACH[1], E. P. HOFFMAN[2], A. I. FADEN[3], B. HARMON[1];
[1]Res. Ctr. Gen Med., Children's Natl. Med. Cr, WASHINGTON, DC; [2]Childrens Natl. Med. Ctr., Washington DC, DC; [3]Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: There have been a number of expression profiling studies of experimental traumatic spinal cord injury, most using microarrays. Microarray data is influenced by amplification and analysis bias, and requires subsequent validation. As an alternative approach to microarrays, we

analyzed original total RNA samples obtained as part of a prior Affymetrix profiling study (GEO GDS2159), with a recently developed multiplex digital quantitation analysis method (NanoString), focused on 800 specific mRNAs associated with inflammation and immunity. Assayed samples were obtained from the region of impact in a C57BL6 mouse model of moderate weight-drop induced contusion injury. Expression of mRNA was evaluated temporally (4 hours -28 days), after injury or parallel sham-injury. After solution-phase hybridization to a molecular barcoded reporter probe, individual mRNAs in the original sample were aligned, immobilized and directly counted. Expression of over 250 inflammation/immune-related mRNAs was increased from 4 hours to 28 days after injury. Pathways or gene families with the greatest number of members represented and highest expression counts (significantly enriched) included: interleukin signaling (IL-1, IL-beta, IL-1R1, IL-1RAP, IL-1R2, IL-18RAP, IL-6, IL-6ST, IL-4R, IL-13RA, IL-2RG, IL-17B), TNF superfamily (TRAIL, TRAIL-R, TNF-alpha, Fas, TNF-R1, TNFSF10, TNFSF11,TNFSF14, caspase 3), cxc and cc subfamily chemokines (CxcL1, CxcL7, Cx3CR1, CcR5, CcL4, CcL3, CcR1 and others), toll-like receptor signaling (TLR1, TLR2, MyD88, IRAK, TLR7/8, NF-kB, caspase 8), cell adhesion and leukocyte-endothelial transmigration (PECAM1, cd40, Cd226, TGAL, TGB2, TGAM, ITGB1, ITGB2, ICAM1, ICAM2, MHC-II, cd80, SELL and others), and natural killer cell cytotoxicity (cd94, FcyRIII, SHP-1). Other pathways that were present but less represented, included those involved in Jak-STAT and MAPK signaling, and B cell and RIG-1 like receptor signaling. These data are in agreement with our previous microarray expression profiling study, which suggested that specific immune response pathways are markedly altered as late as 28 days after moderate contusion injury in the mouse. As the current data reflect direct tabulation of individual mRNAs in the same samples used for the microarrays, they serve as a multiplexed highly parallel validation of the results from the original microarray analysis.

Disclosures: S.M. Knoblach: None. **E.P. Hoffman:** None. **A.I. Faden:** None. **B. Harmon:** None.

Poster

705. Peripheral and Central Nerve Injury

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 705.24/U30

Topic: C.10. Trauma

Support: NJ Comission on Spinal Cord Research

Title: Inflammatory gene expression following spinal cord injury is reduced in osteocalcin null mutant mice

Authors: D. E. BENJAMIN[1], D. SUN[2], *L. A. POHORECKY-DOLINSKY[3], J. VATSON[4], P. PATTERSON-BUCKENDAHL[4];
[1]Cenoxsys Corp., Princeton, NJ; [2]Keck Ctr., Piscataway, NJ; [3]Rutgers Univ., Westfield, NJ; [4]Rutgers Univ., Piscataway, NJ

Abstract: Osteocalcin (OC) is believed to have a role in regulating bone mineralization and may also have extraskeletal functions in energy metabolism. Our recent studies of spinal cord injury (SCI) of OC null mutant (KO) and wild type (WT) mice provided evidence that motor and skeletal recovery were improved in the knockout mice. We are currently investigating the mechanism for protection against SCI afforded by OC deletion. Four OC KO mice and four WT underwent SCI at T9 imposed by the MASCIS contusion procedure. Spinal cord from the injury site was harvested six hours post-injury. RNA was purified using RNeasy protocols and reverse transcribed to cDNA. Gene expression was quantified by RT-PCR with TaqMan probes for several inflammation-related genes. Data revealed a significant decrease in the expression of the IL1b and IL6, NOS2, Arg1, FCGR2A, and CD11b genes, but not TNF-α in OC KO mice. Decreases in these messages suggest that OC gene deletion protects against SCI by a selective anti-inflammatory mechanism. A second set of mice were subjected to hind limb unloading (HLU), a non-traumatic model of musculoskeletal disuse that also affects systemic inflammatory responses. Gene expression analyses revealed greater expression of IL-10 in the lumbar 2 vertebrae of HLU mice that was not affected by OC deletion. The lack of effect of OC gene deletion on IL-10 expression in HLU supports the contention that OC exerts highly specific effects on immune function that can be targeted to potentially mitigate inflammatory disease.
Supported by NJ Commission on Spinal Cord Research.

Disclosures: D.E. Benjamin: A. Employment/Salary (full or part-time):; Cenoxsys Corporation. **L.A. Pohorecky-Dolinsky:** None. **P. Patterson-Buckendahl:** None. **D. Sun:** None. **J. Vatson:** None.

Poster

705. Peripheral and Central Nerve Injury

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Topic: C.10. Trauma

Support: Veterans Administration

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NIH Grant EB014986

Adelson Medical Research Foundation

Title: Transcriptomic profiling of corticospinal neurons after spinal cord injury: Candidate mechanisms of regeneration

Authors: *J. N. DULIN[1], A. BLESCH[2], G. COPPOLA[3], D. H. GESCHWIND[3], M. H. TUSZYNSKI[1,4];
[1]Dept. of Neurosciences, UCSD, La Jolla, CA; [2]Spinal Cord Injury Ctr., Univ. Hosp. Heidelberg, Heidelberg, Germany; [3]UCLA Sch. of Med., UCLA, Los Angeles, CA; [4]Veterans Affairs Med. Ctr., San Diego, CA

Abstract: The corticospinal tract (CST) is the most important motor pathway for voluntary motor control in humans, and it is particularly refractory to efforts to promote its regeneration. This failure of regeneration is in part attributable to a reduced intrinsic growth capacity of these mature neurons. Hence, there remains a great need to identify intrinsic neuronal mechanisms that can be manipulated to promote regrowth of the injured CST. We accordingly sought to gain a greater understanding of the transcriptional mechanisms and downstream signaling networks that are important in axonal regeneration and potentially deficient in corticospinal neurons. To accomplish this, we performed genome-wide screening in: 1) regenerating dorsal root ganglion (DRG) neurons following peripheral nerve conditioning lesions, which enables regeneration of the central branch of these neurons after spinal cord injury, and compared findings to 2) non-regenerating, injured CST neurons following central spinal cord lesions. Analysis of these datasets has led to the identification of transcriptional networks that are similar and divergent in conditioned DRG neurons compared to corticospinal neurons after SCI. These findings suggest that differing populations of adult neurons recruit distinct transcriptional mechanisms after injury that correlate with the success or failure of axonal regeneration. We are currently screening novel candidate mechanisms to determine whether they may enhance corticospinal regeneration.

Disclosures: J.N. Dulin: None. **A. Blesch:** None. **G. Coppola:** None. **D.H. Geschwind:** None. **M.H. Tuszynski:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.01/U32

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH

Title: DISC1 serine-713 phosphorylation-dependent neurodevelopmental switch: Its impact on anatomy, cognition and mental conditions

Authors: *K. ISHIZUKA[1], Y. HORIUCHI[1], S. ISHII[3], N. GAMO[1], A. SAITO[1], T. RATNANATHER[2], S. KULASON[2], D. SCHRETLEN[1], A. KAMIYA[1], M. MILLER[2], H. OKANO[3], A. SAWA[1];
[1]Dept Psychiatry, [2]Ctr. for Imaging Sci., Johns Hopkins Univ., BALTIMORE, MD; [3]Keio Univ., Tokyo, Japan

Abstract: One major limitation that has hindered the progress of research of major mental conditions, such as schizophrenia, is the difficulty in accessing neuronal cells from patients. To overcome this obstacle, we have used cell engineering technology to establish neuronal cell lines from peripheral tissues of human subjects. This strategy is also combined with the use of surrogate tissues and cells that carry some levels of neuronal molecular signatures, such as olfactory neuronal epithelium. We previously reported that a specific phosphorylation of the DISC1 protein, a susceptibility factor for a wide range of mental conditions, determines neural fate during development in animals (Ishizuka et al, Nature, 2011). The goal of this study is to validate whether and how this specific phosphorylation (serine-713 in human DISC1) underlies brain anatomy and function, possibly in association with mental conditions. We have addressed this question using a multi-faceted approach, including human cell biology, brain imaging, and neuropsychological assessment. We found that the levels of DISC1 phosphorylation at serine-713 were markedly reduced in patients with schizophrenia and bipolar disorder with psychotic features compared to controls, when we examined this molecular trait in olfactory immature neuronal cells obtained by quick nasal biopsy. The consistent reduction in patients with schizophrenia was also observed in induced pluripotent stem (iPS) cell-derived neuronal cells. Importantly, the patient neurons differentiated from the iPS cells displayed deficits in neural maturation *in vitro* and *in vivo* (transplantation of human cells into developing rodent brains). Furthermore, we have discovered that the reduction of this specific phosphorylation of DISC1 in biopsied tissues is clearly correlated with smaller volume of middle frontal gyrus and impaired working memory. Thus, this specific molecular signature may be utilized as a predictive marker of several levels of clinical traits associated with major mental conditions, such as schizophrenia.

Disclosures: **K. ishizuka:** None. **Y. Horiuchi:** None. **S. Ishii:** None. **N. Gamo:** None. **A. Saito:** None. **T. Ratnanather:** None. **S. Kulason:** None. **D. Schretlen:** None. **A. Kamiya:** None. **M. Miller:** None. **H. Okano:** None. **A. Sawa:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Intramural Research Program of the National Institute of Mental Health

Fetzer Franklin Fund

Title: MEG comparisons of shared information among schizophrenics, normal controls, and unaffected siblings

Authors: *S. E. ROBINSON[1], A. MANDELL[2], D. R. WEINBERGER[3], R. COPPOLA[1]; [1]MEG Core Group, NIH/NIMH, Bethesda, MD; [2]Psychiatry and Psychotherapy, Univ. of California San Diego, La Jolla, CA; [3]Lieber Inst. for Brain Develop., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Comparisons of shared information for three groups are presented: schizophrenics (n=15), normal controls (n=14) and unaffected siblings (n=10), using symbolic mutual information (SMI) analysis of MEG in a 50-300 Hz bandpass. SMI is a pair-wise non-parametric symbolic measure of mutual information. Data were acquired from a 275-channel MEG (CTF Systems, Inc.) during task-free (rest) and a working memory task (n-back). The most significant SMI differences between schizophrenics and controls show hyper-connectivity in rostral prefrontal cortex (example in Fig 1), whereas the unaffected siblings appear to be hypo-connected in precuneus for a false discovery rate of $q<0.01$. Previous MEG studies, using the n-back task have shown differences among these groups for event related desynchronization of beta-band power in dorsolateral prefrontal cortex and parietal cortex. These SMI results appear to be independent of task or memory work-load. Although group differences are significant further studies are needed to determine the sensitivity and specificity of the SMI measure, and to investigate cofactors such as medication and gender differences.

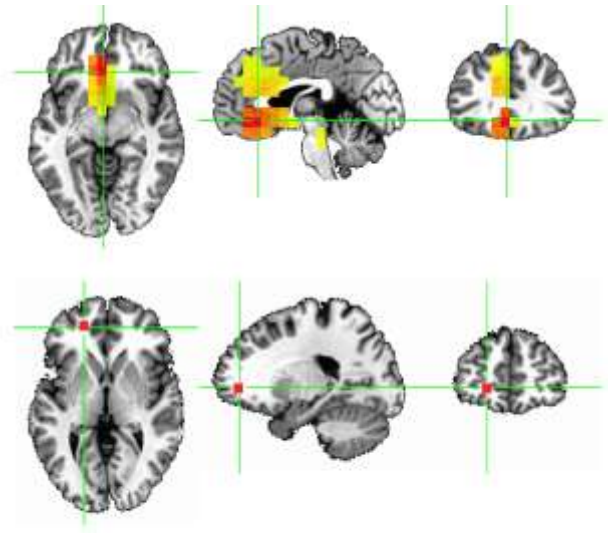

Figure 1: Group difference (top) between schizophrenics (n=15) and normals (n=14) for symbolic mutual information during rest with FDR threshold q<0.01. The seed location is shown at the bottom. This is one example showing hyperconnectivity in rostral prefrontal cortex.

Disclosures: S.E. Robinson: None. **R. Coppola:** None. **A. Mandell:** None. **D.R. Weinberger:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

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Title: Abnormal neural oscillations and lagged connectivity in patients with first episode psychosis

Authors: *A. RAMYEAD[1], E. STUDERUS[1], M. KOMETER[3], U. GSCHWANDTNER[2], P. FUHR[2], A. RIECHER-ROSSLER[1];
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Abstract: Converging evidence suggests that neural oscillations coordinate activity across distributed brain areas, a process that seems to be perturbed in patients with schizophrenia. In particular, neural oscillations in the beta (13-30Hz) and gamma (30-50Hz) frequency bands were consistently found to be disturbed in schizophrenia. However, studies investigating the spatial distribution of these frequencies across brain areas are sparse. Therefore, our goal was to study whether patients with first-episode psychosis (FEP) demonstrate localized abnormal oscillations in both the beta and gamma frequency bands when compared to healthy controls (HC). We hypothesized in particular that in FEP, the connectivity of beta oscillations, the long-range coordinator, would be altered in small-scale networks compared to large-scale networks. As part of the Basel Früherkennung von Psychosen (FePsy) study, 48 FEP and 29 HC were measured using clinical EEG at rest. EEG data were analyzed by the new inverse solution tool eLORETA to assess group differences in current source density of neural oscillations in beta and gamma frequency bands across brain areas. We then assessed lagged connectivity of oscillations in small versus large scale networks using a new, robust and non-biased measure of lagged connectivity. To do so, we fitted a linear mixed-effects model using lagged phase synchronization of the ROI pairs (171 pairs) as the dependent variable and Euclidian distance (within-subjects) and group (between-subjects) along with their interaction as independent variables. Current source density of neural oscillations revealed that FEP patients showed a significantly higher current source density of beta2 (21-30 Hz) and gamma oscillations (30-50Hz) in the left superior frontal cortex compared to HC ($p<.05$, corrected). We also revealed a significant interaction effect between Euclidian distance and group in beta2 lagged connectivity, which was due to a stronger decrease in lagged connectivity with Euclidian distance in the FEP compared to the HC group ($p<.001$, corrected). A heightened activity in the high beta2 and gamma frequency bands in the left superior frontal cortex in FEP patients could potentially reveal the neural underpinnings for an abnormal working memory. Moreover, the significant interaction effect between Euclidian distance and group in beta2 lagged connectivity suggests an apparent impairment in the communication of distributed brain areas in the presence of psychosis.

Disclosures: A. Ramyead: None. **E. Studerus:** None. **M. Kometer:** None. **U. Gschwandtner:** None. **P. Fuhr:** None. **A. Riecher-Rossler:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.04/U35

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Delineating psychotic disorders by use of proton magnetic resonance imaging (^{1}H-MRS)

Authors: *F. HOWELLS, J. H. HSIEH, H. TEMMINGH, D. J. STEIN;
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Abstract: The neurobiological mechanisms that result in the development and persistence of psychotic disorders are currently unclear. Often psychotic disorders are assessed in comparison to healthy controls, in some instance to a second psychotic disorder. In the present study we directly compare three psychotic disorders (schizophrenia, bipolar I disorder with a history of psychotic symptoms, and methamphetamine-induced psychotic disorder) using proton magnetic resonance spectroscopy (^{1}H-MRS), assessing three key brain regions that have been implicated in psychosis: anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (left DLPFC), and thalamus (left thalamus). Our main findings are (1) N-acetyl-aspartate (NAA) and NAA with N-acetyl-aspartyl-glutamate (NAAG) in the ACC is reduced in schizophrenia and methamphetamine-induced psychotic disorder, when compared with healthy controls. (2) NAA with NAAG in the DLPFC is reduced in bipolar I disorder, when compared with healthy controls. (3) Glutamate (Glu) and glutamate with glutamine (Glx) in DLPFC is increased in bipolar I disorder when compared with healthy controls and individuals with methamphetamine-induced psychotic disorder. To our knowledge, this s is the first study that directly compares three psychotic disorders using ^{1}H-MRS. These data are consistent with our previous work in this area, and suggest that similar processes, decreased neuronal integrity and neuronal viability in ACC, may be occurring in schizophrenia and methamphetamine-induced psychotic disorder, with somewhat different processes occurring in bipolar disorder, where there is increased glutamatergic function and decreased neuronal integrity and viability in DLPFC.

Disclosures: **F. Howells:** None. **J.H. Hsieh:** None. **H. Temmingh:** None. **D.J. Stein:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.05/U36

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: National Institute of Mental Health

Hartford Hospital

Canadian Institutes of Health Research

Social Sciences and Humanities Research Council

Title: Amygdala functional connectivity and affective reactivity differentiates manic and depressive mood states across bipolar and unipolar mood disorders

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Abstract: Bipolar disorder (BD) is a severe psychiatric illness characterized by fluctuations between manic, depressive, and euthymic mood states. Despite evidence for strong biological bases of disease, the heterogeneous symptoms associated with BD challenge attempts to accurately diagnose and the treat the disorder. One emerging approach is to examine pathophysiological processes that are specific to BD and distinguish it from other affective disorders, such as responses to emotinally salient information and the regulation of these responses (Philips and Vieta, 2007). Neural differences apparent in bipolar disorder include altered amygdala sensitivity to positive and negative information (Bemophl et al., 2009; Almeida et al., 2010), and disrupted functional connectivity between anterior limbic and medial prefrontal (mPFC) regions (Anand et al., 2009; Anticevic et al., 2013). Here we show that profiles of amygdala response to affectively salient information, along with amygdala-mPFC functional connectivity, further begin to differentiate between mood state among currently manic bipolar (BD-manic; n=9), depressed bipolar (BD-depressed; n=11), and depressed unipolar (MDD-depressed; n=15) adults. All participants performed an Affective Reactivity task in which they viewed blocks of positive, negative, and neutral images (International Affective Picture System: Lang, Bradley, Cuthbert, 2008) during fMRI scanning. Bilateral amygdala response distinguished the BD-manic from the BD-depressed group, such that the BD-depressed group showed significantly greater response to negative versus positive information. Interestingly, no difference was found in bilateral amygdala response to negative versus positive information for the BD-manic and MDD-depressed groups. However, we observed that the BD-depressed and

MDD-depressed groups were further differentiated in task-independent amygdala-mPFC functional connectivity. Together, the findings suggest heightened sensitivity to, and diminished regulation of, negative information during depression across both BD-depressed and MDD-depressed groups. This study suggests that the examination of amygdala reactivity and functional connectivity may begin to help delineate core trans-diagnostic and disorder-specific affective processes.

Disclosures: V. Man: None. **J. Gruber:** None. **D.C. Glahn:** None. **W.A. Cunningham:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.06/V1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH/NIMH Intramural Research Program

Title: The effects of neuroleptic treatment and diagnosis on ventral striatal activation during reward anticipation in drug-free patients with schizophrenia

Authors: *C. HEGARTY[1,2], D. P. EISENBERG[1], J.-C. DREHER[1], P. KOHN[1], J. A. APUD[1], D. R. WEINBERGER[3], K. F. BERMAN[1];
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Abstract: Introduction: Dopamine-driven meso-frontostriatal circuitry has been a focus of cellular as well as neuroimaging studies of schizophrenia and is crucial for reward processing. Reward processing is of particular interest in schizophrenia not only because it relies on dopamine (DA) and frontostriatal networks, but also because clinical aspects of the illness, such as some negative symptoms, may be conceptualized as deficits in reward processing. Past work has shown blunted ventral striatal (VS) activity in schizophrenia during reward processing (Juckel et al. 2006, Morris et al. 2012, Nielsen et al. 2012). We sought to confirm and extend these findings in a larger cohort of patients, carefully maintained on our inpatient ward, both on and off medication during a double-blind, placebo-controlled, counterbalanced study. Methods: In conjunction with fMRI, we used a slow-event-related reward paradigm that was modeled after work in primate midbrain DA cells (Schultz et al. 1997) and enabled us to manipulate the uncertainty of the expected reward. fMRI was conducted on a 3T scanner in 27 medication-free

inpatients (aged 30 + 11 years, 9 women), 19 of whom were studied twice, once after three weeks of placebo and once after three weeks of stable treatment with atypical neuroleptics. Data were analyzed in SPM5 focusing on the striatum and on the highest level of reward uncertainty (50% probability of winning). We compared the 27 drug-free patients to 35 healthy controls, matched for age, sex and race with a voxelwise independent sample t-test, and, with a paired t-test, we compared medicated to drug-free conditions in the patients who completed both arms of the protocol. Results: Between-group analysis demonstrated hypoactivation of the VS in drug-free patients when 50% chance of winning was compared to 25% (x, y, z: 30, 2, -4; $p<0.001$, uncorrected). In patients, neuroleptic treatment blunted the response to 50% uncertainty compared to 0% uncertainty (x, y, z: 32, 7, 6; $p<0.001$, uncorrected). Conclusions: Our findings support past observations of ventral striatal reward processing abnormalities in schizophrenia and suggest that they occur even under medication-free conditions. Neuroleptic treatment may exacerbate these aberrancies in patients, perhaps consistent with the lack of efficacy of these medications in ameliorating the negative symptoms of schizophrenia.

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Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.07/V2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: The Ontario Mental Health Foundation Research Studentship

Title: Evidence for inhibitory deficits in the prefrontal cortex in schizophrenia

Authors: *N. RADHU[1,2], L. GARCIA DOMINGUEZ[2], F. FARZAN[2], M. SEMERALUL[2], M. A. RICHTER[3], Z. J. DASKALAKIS[2];
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Abstract: Abnormal gamma-aminobutyric acid (GABA) inhibitory neurotransmission is a key pathophysiological mechanism underlying schizophrenia (SCZ). Transcranial magnetic

stimulation (TMS) can be combined with electroencephalography (EEG) to index long-interval cortical inhibition (LICI), a measure of GABAergic receptor-mediated inhibitory neurotransmission from the frontal and motor cortex. In previous studies we have reported that SCZ is associated with inhibitory deficits in the dorsolateral prefrontal cortex (DLPFC) compared to healthy subjects and patients with bipolar disorder. These findings provided initial evidence that LICI deficits in the prefrontal cortex may be a pathophysiological marker that is specific to SCZ. The main objective of the current study was to replicate and extend these initial findings by evaluating LICI from the DLPFC in patients with SCZ compared to obsessive-compulsive disorder (OCD). A total of 111 participants were assessed: 38 patients with SCZ (average age: 35.71, 25 males, 13 females), 27 patients with OCD (average age: 36.15, 11 males, 16 females) and 46 healthy subjects (average age: 33.63, 23 females, 23 males)]. LICI was measured in the DLPFC and motor cortex through TMS-EEG. In the DLPFC, LICI was significantly reduced in SCZ compared to healthy subjects ($p = 0.004$) and no significant differences were found between OCD and healthy subjects in the DLPFC ($p = 0.5445$). LICI deficits in the DLPFC were also significantly greater in patients with SCZ compared to patients with OCD ($p = 0.0465$). Finally, there were no significant LICI differences across all three groups in the motor cortex. These results replicate and extend previous findings by demonstrating that LICI deficits in the DLPFC are specific to patients with SCZ and are not a generalized deficit that is shared by many psychiatric disorders of severe psychopathology.

Disclosures: **N. Radhu:** None. **L. Garcia Dominguez:** None. **F. Farzan:** None. **M. Semeralul:** None. **M.A. Richter:** None. **Z.J. Daskalakis:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.08/V3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Michael J. Fox Foundation

CNS Therapeutics

HermoPharma

Title: Complex patterns of activity across the 24-hour day predict susceptibility to mental health and neurodegenerative disorders: Evidence in humans and monkeys

Authors: H. SHOU[1], L. CUI[2], N. HA[3], A. LEROUX[1], K. SUBRAMANIAN[4], Z. ZHANG[5], N. D. RYAN[6], V. ZIPUNNIKOV[1], C. CRAINICEANU[1], *J. L. CAMERON[6], K. MERIKANGAS[2]; [1]Biostatistics, Johns Hopkins Univ., Baltimore, MD; [2]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Neurosci., [4]Neurobio. and Anat., Univ. of Pittsburgh, PITTSBURGH, PA; [5]Neurobio. and Anat., Univ. of Kentucky, Lexington, KY; [6]Univ. Pittsburgh Sch. Med., PITTSBURGH, PA

Abstract: There is increasing interest in how patterns of daily activity are related to mental disorders and susceptibility to developing neurodegenerative disorders. Here we present data on the use of omnidirectional accelerometers (i.e., actigraphy) to collect activity data in monkeys and humans and the use of functional data analysis to examine magnitude and timing of minute-to-minute activity data and functional principal component analysis (fPCA) to examine patterns of activity data collected across two-week periods in both humans (n= 339) and adult female rhesus monkeys (n=30; 15-20 years of age). First, we asked how similar were daily activity patterns in monkeys and humans? fPCA showed that the first four components of the daily activity patterns were very similar in humans and monkeys. Moreover, the 4 highest loading components of monkeys explained 85.44% of the variability in PC1 in humans, and 76.44% of PC2. Conversely, the 4 highest loading components from human explain 85.32% of the variability in PC1 of monkeys, and 83.85% of the variability in PC2 of monkeys. Second, we asked if there were detectable differences in activity patterns in humans with Bipolar disorder? People with Bipolar disorder exhibited altered circadian activity patterns relative to controls with lower average activity in mid to late afternoon (p vs controls < 0.01), and higher day-to-day variation in their activity level (p vs other disorders < 0.03). Third, we asked if differences in activity could predict susceptibility to neurodegenerative disorders? Monkeys were treated with a unilateral, low dose of the neurotoxin, MPTP, to cause a moderate degeneration of nigrostriatal dopamine neurons (a model for Parkinson's disease). Daily variability in PC3 (r=-0.46, p=0.013) and PC4 (r=-0.39, p=0.039) prior to MPTP treatment predicted susceptibility to MPTP measured using the monkey Parkinson's rating scale. These findings suggest that daily patterns of activity provide an important cross-species indicator of brain disorder phenotypes, and that functional data analysis of activity data may provide a powerful method for cross species analysis of susceptibility to mental health and neurodegenerative disorders.

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Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.09/V4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Alterations of mGluR5 and mGluR5 signaling partners in schizophrenia

Authors: *N. MATOSIN[1,3,2], M. ENGEL[1,3,2], F. FERNANDEZ-ENRIGHT[1,3,2], J. S. LUM[1,3,2], J. L. ANDREWS[1,3,2], X.-F. HUANG[1,3,2], K. A. NEWELL[1,3,2];
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Abstract: Animal and genetic studies point towards a role of metabotropic glutamate receptor subtype 5 (mGluR5) in the pathophysiology of schizophrenia, but direct evidence from postmortem schizophrenia studies have been inconsistent. We thus assessed mGluR5 protein levels in samples of human postmortem dorsolateral prefrontal cortex (DLPFC; n=37) and hippocampal cornu ammonis 1 (CA1; cohort subset: n=20) from schizophrenia and matched controls. We also measured the mGluR5 signaling partners, Norbin (neurochondrin), Tamalin (GRASP1), and Preso1 (FRMPD4), which modulate mGluR5 trafficking, localization and recycling. To determine if current antipsychotics influence mGluR5 and mGluR5 signaling partners, lifetime antipsychotic history was correlated with protein measures in postmortem samples. Protein levels were additionally analyzed in rats chronically treated with haloperidol or olanzapine. mGluR5 protein levels were consistently increased in both the DLPFC (22%; $p<0.001$) and CA1 (42%; $p<0.001$) of schizophrenia subjects compared to controls. mGluR5 signaling partners exhibited brain-region dependent alterations, with reductions in the DLPFC (Norbin 37%, $p<0.001$; Tamalin 30%, $p=0.040$; Preso1 29%, $p=0.001$) and increases in CA1 (Norbin 47%, $p<0.001$; Tamalin 34%, $p=0.009$; Preso1 83%, $p<0.001$). There were no effects of current antipsychotics on mGluR5, Norbin, Tamalin or Preso1 in humans or rats. In this study, we provide the first evidence that mGluR5 is increased and mGluR5 signaling partners are differentially altered in two highly important brain regions for schizophrenia. The present findings thus support that mGluR5 regulation is altered in schizophrenia, and that the identified changes in mGluR5, Norbin, Tamalin and Preso1 expression are unaffected by current therapeutics.

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Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.10/V5

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Serotonin transporter clustering in blood lymphocytes predicts the outcome on anhedonia scores in naïve depressive patients treated with antidepressant medication

Authors: *H. J. CARUNCHO[1], T. RIVERA-BALTANAS[3], R. C. AGIS-BALBOA[3], L. E. KALYNCHUK[2], J. M. OLIVARES[3];
[1]Col. of Pharm. and Nutr., [2]Col. of Med., Univ. of Saskatchewan, Saskatoon, SK, Canada;
[3]Biomed. Res. Inst. of Vigo (IBIV), Vigo, Spain

Abstract: In recent reports we have shown that the analysis of clustering of the serotonin transporter (SERT) and the serotonin 2A receptor in blood lymphocytes allows to differentiate two subpopulations of naïve depression patients (D-I and D-II) that will show different therapeutic responses to antidepressant medication as ascertained by the evaluation of symptoms using the Hamilton Depression Rating Scale (HDRS) (Rivera-Baltanas et al., J. Affect. Disord., 2012, 137:46-55; and J. Affect. Dissord., 2014, 163:47-55). However, this scale has some limitations on the measurements of anhedonia symptoms. Anhedonia symptoms are difficult to treat with first-line antidepressants and its presence may represent a predictor of poor treatment response. Therefore in the present study we evaluated the alterations in SERT clustering in lymphocytes with respect to scores in the Self-Assessment Anhedonia Scale (SAAS) in the same populations of D-I and D-II patients that we identified in our previous SERT study (see above). In naïve depression patients there were no differences in SAAS scores between the D-I and D-II groups. However after 8 weeks antidepressant medication patients in the D-I group did not show a significant improvement in SAAS scores ($p>0.836$) or for any of the SAAS subscales, while patients in the D-II group significantly lowered their scores in SAAS ($p<0.002$) and also for all the SAAS subscales (Intensity, $p<0.002$; Frequency, $p<0.04$; Change, $p<0.04$; Physical, $p<0.02$; Intellectual, $p<0.01$; Social, $p<0.05$). Antidepressant treatment induces a significant increase (27%, $p<0.04$) in the number of SERT clusters in lymphocytes only in the D-II group, which prompted us to evaluate a possible correlation between the increase in the number of SERT clusters and the improvement in SAAS scores for D-II patients. We found a negative correlation (increase in number of clusters correlates with a decrease in SAAS scores) for SAAS and the subscales, with the exception of the Change subscale ($p>0.16$): SAAS ($p<0.017$), Intensity ($p<0.04$), Frequency ($p<0.03$), Physical ($p<0.03$), Intellectual ($p<0.03$), Social ($p<0.02$). In conclusion, analysis of SERT clustering in lymphocytes may be consider as a biomarker of antidepressant therapeutic efficacy in anhedonia in depression.

Disclosures: **H.J. Caruncho:** None. **T. Rivera-Baltanas:** None. **R.C. Agis-Balboa:** None. **L.E. Kalynchuk:** None. **J.M. Olivares:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.11/V6

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: K08 NS 52232

R01 NS 70872

CCaTS Career Transition Award to SYC

The Grainger Foundation

Title: Investigating the circuitry effect of nucleus accumbens deep brain stimulation in humans with fMRI

Authors: ***W. GIBSON**[1,2], P. MIN[2,3], O. ABULSEOUD[4], J. FELMLEE[5], K. GORNY[5], S. CHANG[2,3], K. WELKER[5], C. FAVAZZA[5], B. KLASSEN[6], K. LEE[2,3];
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Abstract: Objective: Nucleus Accumbens Deep Brain Stimulation (DBS) is currently being investigated for use in patients with chronic pain and psychiatric disorders such as Obsessive Compulsive Disorder (OCD). Here, we aimed to elucidate the neural circuitry elements which are affected by DBS of the Nucleus Accumbens (NAc) and Ventral Capsule/Ventral Striatum (VC/VS). Methods: This study was approved by the Institutional Review Board at Mayo Clinic and all patients were approved for surgery by the interdisciplinary Mayo Clinic neuromodulation committee. Patients underwent image guided stereotactic implantation of DBS leads using the Schaltenbrand and Wahren atlas. Patients receiving NAc DBS for chronic pain or VC/VS DBS for OCD underwent intraoperative 1.5T fMRI during either initial battery implantation or battery change surgery. This approach involved externalization of the DBS lead and application of blocked stimulus periods using an external pulse generator while simultaneously acquiring gradient echo echo planar imaging. For OCD, a trial monopolar review during awake lead

implantation surgery was performed in order to identify symptom relieving and/or provoking stimulation parameters. Results: For OCD, trial stimulation revealed parameters that caused acute symptom relief, as well as those which induced a sensation that the patient described as “foggy-mindedness." Subsequently, similar stimulation parameters were applied during fMRI. In VC/VS DBS for OCD, fMRI during symptom-relieving stimulation revealed marked negative BOLD signal in a variety of cortical structures including auditory association, prefrontal, insular, and cingulate cortices. Additionally, fMRI results yielded positive BOLD signal in midbrain and thalamic structures and contralateral cerebellum. In contrast, fMRI during stimulation which caused the patient to feel foggy-minded showed positive BOLD signal in prefrontal cortex. During NAc DBS for chronic pain, fMRI revealed widespread negative BOLD in prefrontal, premotor/supplementary motor, insula, cingulate, and visual association cortices. Conclusion: Our results support the hypothesis that NAc DBS results in widespread neural circuit modulation in areas distal to the DBS target and that modulation of these brain structures may underlie the therapeutic effect of nucleus accumbens DBS in chronic pain and in psychiatric disorders.

Disclosures: **W. Gibson:** None. **P. Min:** None. **O. Abulseoud:** None. **J. Felmlee:** None. **K. Gorny:** None. **S. Chang:** None. **K. Welker:** None. **C. Favazza:** None. **B. Klassen:** None. **K. Lee:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.12/V7

Topic: C.19. Drug Discovery and Development

Support: Boehringer-Ingelheim Netherlands

Title: How combined serotonin-1A receptor agonist and 2A-receptor antagonist can heal hypoactive sexual desire disorder (HSDD)

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Abstract: HSDD is a sexual dysfunction in men and women. More than 20% of all women, and probably also men, between 20 and 49 years of age suffer from it. Using PET-scanning we studied differences in brain function between women with no sexual disorder (NHSD) and HSDD women. They watched neutral, and “women friendly” low and high erotic film clips. In

NHSD women, but much less in HSDD women, watching low, but especially high erotic movies resulted in a strong activation involving the parietal, temporal and to a lesser extent prefrontal cortex of the right brain. In contrast, in the NHSD volunteers watching high, but especially low-erotic movies strong de-activation was found in the left temporal and prefrontal cortex. Remarkably, this large area of deactivation did not extend into the medial orbitofrontal cortex (medial Brodmann's area 11). However, this same area was strongly de-activated on both sides in HSDD women, who showed only very small other areas of de-activation. The left hemisphere is thought to be rational and to process established information and control feelings. It also processes speech and writing. The right hemisphere is intuitive, spontaneous, and free with feelings. Both hemispheres receive strong information from the medial orbitofrontal cortex. This study lead to the concept that in NHSD women the medial orbitofrontal cortex, after receiving and assessing the erotic visual information, activates specific regions in the right temporal and frontal lobe and de-activates large areas in the left temporal and frontal lobes. Since the same erotic visual information de-activates the medial orbitofrontal cortex in HSDD women, de-activation in their left temporal and frontal lobes and activation in their right temporal lobe is inhibited, leading to HSDD. It has been reported that an inverse association exists between the desire for social relationships and 5HT2A binding in medial orbitofrontal cortex. In animals the highest receptor density for 5-HT2A and 5-HT1A receptors was found in the frontal cortex. In isolation-reared rats in this same frontal cortex the 5-HT2A receptor binding site densities were significantly increased, while 5-HT1A receptor binding site densities were significantly reduced. A similar situation might exist in HSDD men and women, increased 5-HT2A and decreased 5-HT1A receptor binding site density. It would explain why flibanserin, a serotonin-1A receptor agonist and 2A-receptor antagonist, restores the activation level of the medial orbitofrontal cortex in the HSDD men and women by reducing the 5-HT2A receptor binding sites and increasing the 5-HT1A receptor binding sites, solving their sexual and possibly other emotional problems.

Disclosures: G. Holstege: None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.13/V8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH83862

MH94888

MH64168

MH40210

The American Foundation for Suicide Prevention

Diane Goldberg Foundation

Title: Lithium increases granule neuron number in the dentate gyrus of subjects with bipolar disorder

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Abstract: Bipolar disorder (BD) is one of the most life-threatening mood disorders. However the discovery of lithium (Li+) as a mood-stabilizer revolutionized the treatment of BD and is now used in psychiatry as a mainstay for BD treatment. Lithium has acute anti-manic and anti-depressant effects, long-term prophylactic effects, and anti-suicidal effects. The therapeutic mechanisms of Li+ remain to be fully elucidated, partly because of an array of potential targets of action. Chronic Li+ treatment up-regulates cell survival molecules (e.g., Bcl-2 and BDNF) while down-regulating pro-apoptotic molecules (e.g., Bax and caspase), thus preventing or even reversing neuronal cell death. In the rodent, Li+ up-regulates neurogenesis and the survival of newborn cells in the hippocampus. We sought to determine the number of neural progenitor cells (NPCs) in the dentate gyrus (DG) of subjects diagnosed with BD who were treated with Li+, compared to untreated patients and non-psychiatric controls (NC), to test the hypothesis that Li+ has a neurotrophic effect on the human hippocampus. We studied 5 NC, 14 untreated subjects with BD, and 4 BD subjects treated with Li+ (BD*Li). All subjects died suddenly and had no neurological disease. After completion of the psychological autopsy, toxicology, and neuropathological examination, whole frozen hippocampi were fixed, sectioned and immunostained for neuronal nuclear antigen to identify mature granule neurons (GNs) in the dentate gyrus. Stereological cell counting was performed using unbiased stereology (StereoInvestigator, MBF Inc.). Groups showed different number of mature GNs in the anterior ($p=.021$) DG compared with untreated BD subjects and controls. Subjects treated with Li+ had more GNs in anterior DG than untreated BD subjects ($p=.017$) and did not differ from controls. Groups did not differ for age or PMI, but groups did differ for Global Assessment Scale (GAS) score ($p<.001$), which was higher in untreated BD subjects than in BD treated with lithium and controls. Lithium treatment is associated with more dentate gyrus granule cells in subjects with

bipolar disorder, and BD subjects treated with lithium have a greater number of GNs in anterior DG that is comparable to that found in controls with no psychiatric disease. This suggests lithium may affect DG cell maturation and survival in human as was shown by preclinical studies. Subjects treated with lithium also had better global functioning, opening the possibility that DG cell viability has a role in symptom improvement in bipolar disorder.

Disclosures: **A.N. Santiago:** None. **T.H. Butt:** None. **G.B. Rosoklija:** None. **A. Dwork:** None. **R. Hen:** None. **V. Arango:** None. **J. Mann:** None. **M. Boldrini:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.14/V9

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH MH092301

Title: Intrinsic activity in cortico-limbic networks predicts ECT response in major depression

Authors: ***A. M. LEAVER**, T. PIRNIA, S. JOSHI, R. WOODS, R. ESPINOZA, K. NARR; UCLA, Los Angeles, CA

Abstract: Converging evidence indicates that major depressive disorder (MDD) is associated with disruptions in cortico-limbic networks. Consequently, studies of functional connectivity can be particularly informative in identifying those networks affected by MDD and its treatment. In the current study, we measured intrinsic neural activity in MDD patients receiving electroconvulsive therapy (ECT), which can elicit a relatively rapid improvement in MDD symptoms relative to standard therapies (i.e., weeks vs. months), making it ideal for longitudinal research. Using independent component analysis (ICA) of fMRI data, we assessed changes in "resting state" networks (RSNs) involving structures widely implicated in depression, specifically medial cortical and subcortical structures, as well as the medial and anterior temporal lobes. We measured changes in functional connectivity in these RSNs associated both with symptom improvement and with ECT regardless of treatment outcome. During an already prescribed course of ECT, patients were assessed: 1) before ECT, 2) after 2 treatments, and 3) after depressive symptoms had stabilized, approximately 3-4 weeks after beginning ECT. Age- and sex-matched controls were scanned twice, also approximately 3-4 weeks apart, for comparison. After ECT, consistent changes in functional connectivity with MDD-relevant RSNs

were seen in several regions, including: right anterior temporal cortex, mediodorsal thalamus, ventral striatum, and subgenual anterior, dorsal, and posterior cingulate cortices. Post hoc ROI analyses showed that these effects were not strongly correlated with treatment outcome, and that these regions were highly intercorrelated both in controls and MDD patients. Thus, these regions could reflect points of contact between the seizure activity elicited during ECT (typically originating from right-temporal stimulation) and MDD-relevant RSNs. Symptom improvement, by contrast, was associated with more circumscribed effects involving connectivity between MDD-related RSNs and anterior cingulate and ventromedial prefrontal cortex. This suggests that although the effects of ECT itself may be more widespread, functional changes within medial prefrontal cortex may be more relevant for treatment outcome. Neurostimulation therapies that are better able to directly modulate medial prefrontal activity may be a target for future research.

Disclosures: **A.M. Leaver:** None. **K. Narr:** None. **T. Pirnia:** None. **R. Woods:** None. **R. Espinoza:** None. **S. Joshi:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 706.15/V10

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: CAPES

FAPERGS

CNPq

Title: Accelerated immunosenescence in patients with bipolar disorder type 1

Authors: *L. B. RIZZO[1], C. H. DO PRADO[2], A. WIECK[2], B. L. CORREA[2], R. P. LOPES[3], *. GRASSI-OLIVEIRA[4], E. BRIETZKE[5], M. E. BAUER[2];
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Abstract: Background: Bipolar disorder (BD) is a mood disorder characterized by dysruptive states of mania and depression. BD has been associated with immunological disbalance, characterized by low-grade inflammation and immune activation. The processes of aging of the

immunological system (immunosenescence) is characterized by an increase in a senescent lymphocyte population (CD8+CD28-), reduction of T regulatory cells (Treg) and short telomere length (TL), which lead to low-grade inflammation. Also, the human cytomegalovirus (CMV) has been implicated with accelerating immunosenescence. Since BD is associated with immunological changes, we decided to evaluate immunosenescence in BD type I patients. Aim: Evaluate immunosenescence markers in women with BD type I. Methods: Twenty-seven euthymic female subjects with BD type I and 24 age- and sex-matched controls were recruited in this study. Lymphocytes were isolated and stimulated *in vitro* to assess Th1/Th17/Th2 cytokines (IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ and TNF-α). Lymphocyte subsets and cytokines were assessed by multi-color flow cytometry. TL was measured by qPCR and CMV titers were assessed by chemiluminescent assays. Results: BD patients had reduced proportions of natural Tregs (CD4+ CD25+ FoxP3+) ($p<0.01$) and higher senescence-associated cells (CD8+ CD28-) in BD ($p<0.0001$) in relation to healthy controls. Besides, cytokine production was elevated (all $p<0.01$), with a Th1 trend. BD patients had shorter TL but increased CMV-IgG levels than controls (all $p < 0.01$). The CMV-IgG levels were inversely correlated with TL and positively CD8+CD28- T cells. Conclusions: The data concur to the hypothesis early accelerated aging in BD as shown by shortened telomeres and expansion of senescent T cells. This study also indicates that CMV infection may be a driving force in the process of early immunosenescence in BD.

Disclosures: L.B. Rizzo: None. **C.H. do Prado:** None. **A. Wieck:** None. **B.L. Correa:** None. **R.P. Lopes:** None. ***. Grassi-Oliveira:** None. **E. Brietzke:** None. **M.E. Bauer:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

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Program#/Poster#: 706.16/V11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: R21 MH094781

R21 MH094781 S1

Title: Pretreatment resting state salience network connectivity predicts response to psychotherapy in major depression

Authors: *A. J. CROWTHER[1], J. MINKEL[3,2], T. MOORE[3], M. SMOSKI[3], G. DICHTER[2,3,4]; [2]Psychiatry, [1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Dept. of Psychiatry and Behavioral Sci., Duke Univ. Med. Ctr., Durham, NC; [4]Duke-UNC Brain Imaging and Analysis Ctr. (BIAC), Durham, NC

Abstract: Background- Major Depression (MDD) is the second leading cause of disability worldwide yet there are no prospectively validated biomarkers of antidepressant treatment response, which hinders treatment efficiency. A number of research groups have reported abnormal resting state functional connectivity measured by fMRI (rs-fcMRI) in MDD, but no research to date has examined whether rs-fcMRI may predict response to psychotherapy in MDD. In this study we tested whether pretreatment seed-based rs-fcMRI was predictive of treatment response to psychotherapy in patients with MDD. Methods- Using functional magnetic resonance imaging, we scanned 24 outpatients with MDD and 20 matched healthy controls. Subjects performed a pretreatment resting state functional MRI session prior to completing a course of Brief Behavioral Activation Therapy for Depression (BATD) for up to 12 weeks. Whole brain voxel-wise rs-fcMRI was performed using 9 seed regions representing 5 resting state networks: the Default Mode Network (DMN); the Salience Network (SN); the Dorsal Attention Network (DAN); and the Executive Control Network. MDD vs. Control group comparisons showed significant voxel cluster differences in connectivity (cluster corrected $p<.005$) and average cluster values were extracted at the subject-level for treatment correlations. Results- Between-group analysis revealed the following: Within the DMN, we found increased rs-fcMRI in the MDD group between the posterior cingulate and the left middle temporal cortex. Within the DAN, we found decreased rs-fcMRI in the MDD group between the left intraparietal sulcus and left orbital frontal cortex. Within the SN, we found that the MDD group displayed decreased rs-fcMRI of the left insula and right insula with the left middle temporal cortex along with increased rs-fcMRI of the right insula with the left occipital cortex. Of the significant between-group rs-fcMRI findings, right insula-left occipital cortex hyperconnectivity was negatively correlated with treatment response ($p=0.0088$). Conclusions- We found functional connectivity abnormalities in multiple resting state networks in MDD. Convergence of cluster location across the DMN and SN suggests that the middle temporal cortex may be a hub of altered connectivity in MDD. Additionally, baseline right insula-occipital cortex connectivity predicted response to psychotherapy in the MDD group. To our knowledge this is the first study to evaluate a resting state rs-fcMRI predictor of psychotherapy response in MDD. Future research will focus on the utility of this endophenotype for predicting response to psychotherapy and other antidepressant interventions.

Disclosures: A.J. Crowther: None. **J. Minkel:** None. **T. Moore:** None. **M. Smoski:** None. **G. Dichter:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

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Program#/Poster#: 706.17/V12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: CREST

Title: Transcriptomic evidence for immaturity of the prefrontal cortex in patients with schizophrenia

Authors: *H. HAGIHARA[1,2], K. OHIRA[1,2], K. TAKAO[2,3], T. MIYAKAWA[1,2,3];
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Abstract: Schizophrenia, a severe psychiatric disorder, has a lifetime prevalence of 1%. The exact mechanisms underlying this disorder remain unknown, though theories abound. Recent studies suggest that particular cell types and biological processes in the schizophrenic cortex have a pseudo-immature status in which the molecular properties partially resemble those in the normal immature brain. However, genome-wide gene expression patterns in the brains of patients with schizophrenia and those of normal infants have not been directly compared. Here, we show that the gene expression patterns in the schizophrenic prefrontal cortex (PFC) resemble those in the juvenile PFC. We conducted a comparative analysis using microarray data sets derived from the dorsolateral PFC (DLFC) of patients with schizophrenia and the DLFC of developing normal human brains, revealing a striking similarity. The results were replicated in a second DLFC data set and a medial PFC (MFC) data set. We also show that more than half of genes representing transcriptional immaturity of schizophrenic PFC were developmentally regulated in fast-spiking interneurons, astrocytes, and oligodendrocytes. These results may imply the pseudo-immaturity of these cell types in schizophrenic PFC. To test whether medications, which often confound the results of postmortem analyses, effect on the juvenile-like gene expressions in schizophrenic PFC, we compared the gene expression patterns showing transcriptomic immaturity of the schizophrenia PFC with those of rodent PFC treated with antipsychotic drugs. The results showed no apparent similarities between the two conditions, suggesting that the juvenile-like gene expression patterns observed in the schizophrenic PFC could not be accounted for by medication effects. The developing human PFC showed a gene expression pattern similar to that of the PFC of naive Schnurri-2 knockout mice, an animal

model of schizophrenia with good face and construct validity. This result also supports the idea that the transcriptional immaturity in of the schizophrenic PFC is not due to medication effects. Collectively, our results provide evidence that pseudo-immaturity of PFC resembling that of the juvenile brain may be an endophenotype for schizophrenia.

Disclosures: H. Hagihara: None. **K. Ohira:** None. **K. Takao:** None. **T. Miyakawa:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

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Program#/Poster#: 706.18/V13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 5R01 NS070009-03

K01 MH077955-05

Title: Pgc-1α-mediated transcriptional regulation in pyramidal neurons impacts electrophysiological properties and behavioral output

Authors: *L. J. MCMEEKIN, A. S. BOHANNON, J. J. HABLITZ, R. M. COWELL; Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: The transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) has been implicated in multiple psychiatric and neurodegenerative diseases, however its cell-specific roles in transcriptional regulation are not wholly understood. In the brain, this coactivator is highly expressed within GABAergic interneurons. Our lab has found that while PGC-1α does play a distinct role within this cell population, specifically in parvalbumin-positive interneurons, this coactivator has a key role in regulating gene programs within pyramidal neurons of the cortex and hippocampus as well. Mice lacking PGC-1α in these pyramidal neuron populations were generated using cre-lox technology. Mice expressing cre-recombinase driven by the EMX1 promoter were crossed to those expressing loxP sites flanking exons 3-5 of the *PPARCG1A* gene; transcriptional, electrophysiological, and behavioral data was collected at postnatal day 90. Loss of PGC-1α within pyramidal neurons of the somatosensory cortex leads to reductions in genes involved in synchronous neurotransmitter release (synaptotagmin-2, Syt2), structural maintenance (neurofilament heavy chain, Nefh), and metabolism (phytanoyl-CoA hydroxylase, Phyh). Further, preliminary whole-cell patch clamp

data indicate an increased rheobase suggesting reduced excitability when PGC-1α is absent. At the behavioral level, animals lacking PGC-1α in pyramidal neurons exhibit hyperactivity in an open field primarily within the first ten to fifteen minutes of a thirty minute session, indicating an impaired ability to acclimate to a novel environment. The implications of these findings are relevant to psychiatric and neurological disorders marked by disruption in excitatory:inhibitory balance of projection neurons and cognitive and/or motor dysfunction. Additional studies are being conducted to investigate the role of PGC-1α within this cell-type and others, specifically striatal medium spiny neurons, downstream targets of cortical pyramidal neurons, using cre-recombinase driven by the RGS9L promoter. These data may elucidate the way in which PGC-1α-mediated transcriptional regulation facilitates between-region communication for directed motor output.

Disclosures: L.J. McMeekin: None. **A.S. Bohannon:** None. **J.J. Hablitz:** None. **R.M. Cowell:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

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Program#/Poster#: 706.19/V14

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH066123

Title: Mapping dopaminergic deficiencies in the substantia nigra/ ventral tegmental area in schizophrenia

Authors: *E. PEREZ-COSTAS, M. W. RICE, R. C. ROBERTS, M. MELENDEZ-FERRO; Dept. of Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, BIRMINGHAM, AL

Abstract: Previous work from our laboratory supports that deficits in tyrosine hydroxylase primarily affect diencephalic regions of the substantia nigra/ventral tegmental area (SN/VTA) in schizophrenia. The aim of this study was to assess if tyrosine hydroxylase (TH) deficits could be linked to significant neuronal loss, and to qualitatively map these deficits. We performed neuronal counts to test: 1) if there was a significant reduction in the total number of neurons in the SN/VTA in schizophrenia; and 2) if the ratio dopaminergic/total neurons, was significantly reduced. Neuronal counts were analyzed independently for the diencephalic (i.e. rostral) and

mesencephalic (i.e. mid-caudal) sub-regions, and also for the entire rostro-caudal extent of the SN/VTA. We did not find any significant differences in the total number of neurons, dopaminergic neurons, or their ratio. These data support that the dopaminergic deficits previously reported cannot be explained by a reduction in the number of neurons. Our qualitative study of TH expression showed a marked decrease in labeling of neuronal processes and cell bodies, which was subregion-specific. Dorsal diencephalic dopaminergic populations of the SN/VTA presented the most conspicuous decrease in TH expression. These data support the existence of pathway-specific dopaminergic deficits that would affect the dopamine input to the cortex.

Disclosures: E. Perez-Costas: None. **M.W. Rice:** None. **R.C. Roberts:** None. **M. Melendez-ferro:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

Location: Halls A-C

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Program#/Poster#: 706.20/V15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: DISC1 regulates endoplasmic reticulum calcium dynamics

Authors: S. PARK[1], J. JEONG[1], Y.-U. PARK[1], K.-S. PARK[1], H. LEE[1], N. LEE[1], S.-M. KIM[1], C. PARK[1], Y. SEO[1], B. SEO[1], K. KURODA[2], M. NGUYEN[3], K. KAIBUCHI[2], *S. PARK[1]; [1]Life Sci., POSTECH, Pohang, Korea, Republic of; [2]Nagoya Univ., Nagoya, Japan; [3]Univ. of Calgary, Calgary, AB, Canada

Abstract: Disrupted-in-schizophrenia-1 (DISC1) has emerged as a convincing susceptibility gene for multiple mental disorders, but its mechanistic link to the pathogenesis of schizophrenia related psychiatric conditions is not completely understood. It has been suggested that a complete understanding of the cellular functions of DISC1 may provide important insight into the molecular basis of schizophrenia. Here, we show that DISC1 localizes to the endoplasmic reticulum (ER). EXOC1, a subunit of the exocyst complex, interacted with DISC1 and affected its recruitment to one of the ER. Abnormal ER calcium responses were observed in hippocampal neurons from DISC1-deficient mutant mice. Moreover, perturbation of ER calcium dynamics upon DISC1 knockdown was effectively reversed by treatment with antipsychotic drugs, such as clozapine and haloperidol. These results collectively indicate that DISC1 is a regulatory factor in

ER calcium dynamics, and support a potential link between a perturbed intracellular calcium signaling and schizophrenia pathogenesis.

Disclosures: **S. Park:** None. **S. Park:** None. **J. Jeong:** None. **Y. Park:** None. **K. Park:** None. **H. Lee:** None. **S. Kim:** None. **N. Lee:** None. **C. Park:** None. **Y. Seo:** None. **B. Seo:** None. **K. Kuroda:** None. **M. Nguyen:** None. **K. Kaibuchi:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Schizophrenia Research Institute

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NHMRC

NIH (NIAAA) R24AA012725

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Title: Reduced cortical grey matter volume in patients with schizophrenia is exaggerated in those with high inflammatory cytokines

Authors: *V. S. CATTS[1,2,3], Y. ZHANG[1,2,3], D. SHEEDY[4], J. KRIL[4], C. SHANNON WEICKERT[1,2,3];
[1]Schizophrenia Res. Lab., Neurosci. Res. Australia, Randwick, Sydney, Australia; [2]Schizophrenia Res. Inst., Sydney, Australia; [3]Sch. of Psychiatry, Univ. of New South Wales, Sydney, Australia; [4]Discipline of Pathology, Univ. of Sydney, Sydney, Australia

Abstract: Many *in vivo* imaging studies have demonstrated cortical grey matter volume deficits in patients with schizophrenia, but only few studies have measured volumes in postmortem brain tissue from patients with schizophrenia. The first objective of our study was to quantify grey

matter, white matter and regional cortical volumes in a collection of postmortem brains from patients with schizophrenia and matched controls. Next, we wanted to test if the subset of patients with schizophrenia we previously defined as “high inflammation subgroup” had less brain volume as compared to individuals without inflammation. Following 14 days in 15% buffered formalin, hemispheres from 47 patients with schizophrenia (31 males, 16 females; mean age 52.6±13.7 years) and 45 control individuals (31 males, 14 females; mean age 51.9±13.5 years) were sectioned at 3 mm in the coronal plane and photographed with a scale bar. Brain volumes (total hemisphere, cortical grey matter) were quantified using Cavalieri’s probe. Increased expression of inflammatory cytokines in prefrontal cortex was found in about 40% of people with schizophrenia as previously described (Fillman et al. 2013 Mol Psychiatry 18:206-214). We found that total hemisphere volume correlated negatively with age and positively with brain weight. Men had significantly larger brains, as measured both by hemisphere volume ($F(1,87)=47.354$, $p<0.001$, co-varied for age) and brain weight ($F(1,89)=48.70$, $p<0.001$, co-varied for age). We found a significant reduction in cortical grey matter/total hemisphere volume (2%) in patients with schizophrenia compared with controls ($F(1,86)=6.201$, $p=0.015$, co-varied for age). The “high inflammation” subgroup of patients was associated with a significantly smaller cortical grey matter/total hemisphere volume ratio relative to patients with low inflammation and unaffected controls (2.9-3.8%: $F(1,43)=5.336$, $p=0.026$, co-varied for age). We conclude that reduced cortical volume is evident in a postmortem brain tissue collection from patients with schizophrenia and that the reduction appears to be particularly associated with a previously identified subtype of schizophrenia defined by high expression of inflammatory cytokines and gliosis. Our studies suggest that degenerative events may characterize the schizophrenia brain and that some mild form of tissue loss may occur in a subset of people with schizophrenia. Future studies will seek to determine if the cortical volume loss is specific to certain cortical regions and to relate the volume deficits to other cellular and molecular factors associated schizophrenia neuropathology.

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Poster

706. Psychosis and Affect: Human Biomarker Studies

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH066123

Title: Cytochrome c oxidase dysfunction in the substantia nigra/ ventral tegmental area in schizophrenia

Authors: *M. MELENDEZ-FERRO, M. W. RICE, K. L. SMITH, R. C. ROBERTS, E. PEREZ-COSTAS;
Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, BIRMINGHAM, AL

Abstract: Metabolic anomalies are a well-documented but complex facet of schizophrenia pathology. Optimal cellular performance requires the proper functioning of the electron transport chain, which is constituted by four enzymes located within the inner membrane of mitochondria. These enzymes create a proton gradient that is used to power the enzyme ATP synthase, producing ATP, which is crucial for the maintenance of cellular functioning. Anomalies in a single enzyme of the electron transport chain are sufficient to cause disruption of cellular metabolism. The last of these complexes is the cytochrome c oxidase (COX) enzyme, which is composed of thirteen different subunits. COX is a major site for oxidative phosphorylation, and anomalies in this enzyme are one of the most frequent causes of mitochondrial pathology. The objective of the present report was to assess if metabolic anomalies linked to COX dysfunction may contribute to substantia nigra/ventral tegmental area (SN/VTA) pathology in schizophrenia. We tested COX activity in postmortem SN/VTA from schizophrenia and non-psychiatric controls. We also tested the protein expression of key subunits for the assembly and activity of the enzyme, and the effect of antipsychotic medication on subunit expression. COX activity was not significantly different between schizophrenia and non-psychiatric controls. However, we found significant decreases in the expression of subunits II and IV-I of COX in schizophrenia. Interestingly, these decreases were observed in samples containing the entire rostro-caudal extent of the SN/VTA, while no significant differences were observed for samples containing only mid-caudal regions of the SN/VTA. Finally, rats chronically treated with antipsychotic drugs did not show significant changes in COX subunit expression. These findings suggest that COX subunit expression may be compromised in specific sub-regions of the SN/VTA (i.e. rostral regions), which may lead to a faulty assembly of the enzyme and a greater vulnerability to metabolic insult.

Disclosures: M. Melendez-Ferro: None. **M.W. Rice:** None. **K.L. Smith:** None. **R.C. Roberts:** None. **E. Perez-costas:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH 071533

T32 MH 16804

P30CA047904

Title: Protein expression distinguishing layers 3 and 5 of the human prefrontal cortex

Authors: *M. L. MACDONALD[1], D. ARION[1], M. GRUBISHA[1], D. A. LEWIS[1], N. YATES[2], R. SWEET[1];
[1]Psychiatry, [2]BioMedical Mass Spectrometry Ctr., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: There is now substantial evidence for morphological alterations of dendrites and loss of dendritic spines of glutamatergic neurons in the cerebral cortex of subjects with schizophrenia, with layer 3 particularly affected. Because disruptions of glutamate signaling have been shown to induce similar morphologic disturbances in model systems, it would be highly desirable to investigate glutamate signaling pathways with laminar specificity in the brain tissue of patients. Laser capture microdissection followed by linear amplification allows for quantification of mRNA expression with laminar specificity, while immunohistochemistry/confocal microscopy allows for the investigation of a handful of proteins with high spatial resolution. However, there is not currently an approach for investigating large numbers of proteins in specific cortical layers. Here we present a Laser Capture Microdissection - targeted Mass Spectrometry approach for the quantification of hundreds of proteins within individual cortical layers of human postmortem tissue. This method was used to compare the expression of 160 proteins between dorsolateral prefrontal cortex layers 3 and 5 from three individuals. Protein quantification by targeted Mass Spectrometry in individual layers collected by laser capture microdissection (4.5E7 μm3) was highly reproducible and linear. The mean CV between samples from three subjects was 8.5%, with 75% of the proteins assayed having a CV ≤ 10%. A dilution curve for PSD95, generated from increasing areas of captured tissue, had an R2 of 0.97. Unsupervised hierarchal clustering by protein expression distinguished between layers 3 and 5. The expression of the vast majority of proteins, normalized to captured layer volume, was higher in layer 3 compared to layer 5. Expression of most ionotropic glutamate receptor subunits was similar between the two layers, while metabotropic glutamate receptors were higher in layer 3. The expression of CAMKIIA and Gprotein subunits was also higher in layer 3. Experiments to increase both the number of subjects and proteins assayed are ongoing.

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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.; Dr. Lewis currently receives investigator-initiated research support from the Bristol-Myers Squibb Foundation, Bristol-Myers Squibb, Curridium Ltd, and Pfizer.. **N. Yates:** None. **R. Sweet:** None.

Poster

706. Psychosis and Affect: Human Biomarker Studies

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 5K01MH077955-05

Title: The expression of developmentally-regulated PGC-1α-dependent genes is reduced in the cortex of patients with schizophrenia

Authors: *R. M. COWELL[1], E. K. LUCAS[3], J. MOLINA[4], J. H. MEADOR-WOODRUFF[1], J. E. KLEINMAN[5], R. E. MCCULLUMSMITH[6], R. C. HENDRICKSON[2], R. C. ROBERTS[1], K. L. GAMBLE[1], L. J. MCMEEKIN[1];
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Abstract: PGC-1α (peroxisome proliferator-activated receptor-gamma coactivator 1-alpha) is a transcriptional coactivator expressed in tissues with high metabolic demand. Several reports have linked PGC-1α to psychiatric disorders including anxiety disorder, bipolar disorder, and schizophrenia, but its involvement in the pathophysiology of these disorders is unclear. Previous experiments utilizing whole body and neuron-specific PGC-1α knockout mice revealed a set of cortical PGC-1α-dependent transcripts involved in calcium buffering (parvalbumin - PV), synchronous neurotransmitter release (synaptotagmin 2 - Syt2; complexin 1 - Cplx1) and axonal integrity (neurofilament heavy chain - Nefh). Here, we show that patients with schizophrenia exhibit reductions in the expression of all four PGC-1α-dependent genes in the anterior cingulate cortex (n = 33/group), which is not recapitulated in rats treated chronically with the antipsychotic drug haloperidol. While control subjects with high PGC-1α expression exhibit high PV and Nefh expression, patients with schizophrenia do not, suggesting a disrupted association between the expression of PGC-1α and its targets in schizophrenia. In fact, in the same samples, we found a reduction in the expression of nuclear respiratory factor 1 (Nrf1), a PGC-1α-interacting

transcription factor with multiple putative binding sites in the proximal promoters of PV, Syt2, Cplx1, and Nefh. To determine the potential cell types affected by changes in these transcripts in human brain, we evaluated their transcriptional expression profile throughout normal human cortical development (n=269) and performed double-labeling immunofluorescence experiments for Syt2, Cplx1, and Nefh in normal human prefrontal cortex and neuron-specific markers. All transcripts increased significantly between birth and early adulthood in normal postmortem human cortex samples, and while Cplx1, Syt2, and Nefh immunoreactivity overlapped with PV in the cell bodies and processes of cortical interneurons, Syt2 and Nefh were also found in excitatory neurons and synapses. These data suggest that schizophrenia involves a disruption in a PGC-1α-associated developmental transcriptional program in multiple cortical cell types and that approaches to enhance PGC-1α activity or the activity of associated transcriptional regulators could restore normal maturation-related gene programs and potentially improve cortical function.

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Poster

706. Psychosis and Affect: Human Biomarker Studies

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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Title: Function of GluN receptors in pyramidal neurons is hypoactive in schizophrenia

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Abstract: NMDA receptor (GluN) hypofunction causes divergent abnormalities on microcircuit activity depending on its cellular locus, *i.e.,* pyramidal cells vs. interneurons. As such, the cellular locus of receptor hypofunction is a critical question to be addressed in the GluN hypofunction hypothesis of schizophrenia (SCZ). Receptor function in specific cell types is typically examined using electrophysiology, which offers detailed readouts of the functional nature of receptors and their impact on circuitry. It is difficult however to assess the complex molecular events underlying receptor function in these studies. To address this, we have devised an approach that can capture GluN complexes that are specific to subtypes of neurons. Our approach is based on immunoprecipitation of protein extracts using protein as bait that is specific to the cell types as well as integral to GluN complexes. We applied this approach to postmortem brains of patients with schizophrenia and controls to examine whether molecular signature of GluN activity is altered in GluN complexes derived from pyramidal neurons in schizophrenia using CAKIIa as bait. CAMKIIa is expressed almost exclusively in pyramidal neurons in various species and serves a critical role in NR complexes. We examined the expression of CAMKIIa in the DLPFC using immunohistochemistry, in which CAMKIIa was not co-localized with PV, calretinin or calbindin. Immunoprecipitation of synaptic membrane extracts with antibodies for CAMKIIa, when combined with subsequent immunoprecipitation for GluN in particular, capture NR complexes that are specific to cell types that express CAMKIIa. Previously, we have observed that both basal and ligand induced association of NR with PKCγ is decreased in SCZ. The PKCγ-NR signaling pathway is likely restricted to pyramidal cells, because somatal PKCγ in the cerebral cortex and hippocampal formation has only been detected in pyramidal cells. In this study, we examined dorsolateral prefrontal cortices from 12 patients and their age-sex matched controls for GluN activation mediated by NMDA and Glycine. Tissue extracts were immunoprecipitated with antibodies for CAMKIIa; NMDA/glycine induced lesser activation of of CaMKIIα (pT^{286}) in the SCZ cases compared to controls ($p=0.018$, $t=3.43$, $df=11$). In addition, we found decreases in src ($p=0.052$, $t=2.028$, $df=11$) and NR1($p=0.067$, $t=2.028$, $df=11$) in CaMKIIα containing GluN complexes derived from the patients group. The results of this study offer direct evidence that the function of GluN complexes is decreased in pyramidal neurons of the DLPFC in SCZ cases compared to controls.

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Poster

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Title: Inhibitory interneuron deficit is related to elevated death receptor expression in the orbital frontal cortex in schizophrenia

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Abstract: Cortical inhibitory interneuron deficit is one of the most consistent forms of neuropathology known to contribute to the development of schizophrenia. It is still unclear if reductions in inhibitory interneuron mRNAs [particularly somatostatin (SST) and parvalbumin (PV)] are related to cell death in people with schizophrenia. Recently, we provided neurobiological evidence showing reductions in GAD67 and Dlx mRNAs in the orbital frontal cortex (OFC) in schizophrenia. Here we determine if these inhibitory deficits are contributed by SST and/or PV. We test if mRNAs encoding cell-death signalling molecules (FASR; TNFSF13) relate to SST and PV mRNA expression in the OFC in people with schizophrenia. Inhibitory interneuron mRNAs (SST; PV: *in situ* hybridisation, qPCR) and death receptor signalling mRNAs (FASR; TNFSF13: qPCR) were measured in control (n=38) and schizophrenia (n=38) subjects. SST mRNA+ interneuron-like cells were quantified in layer II in the gyrus rectus (OFC). Gray matter SST and PV mRNA levels were correlated with interstitial white matter neuron density (GAD65/67, NeuN), and death signalling mRNAs. SST expression was reduced in OFC layers I-VI in schizophrenia (both *in situ* and qPCR), with greatest deficit in layer II (67%). SST mRNA+ neuron density was reduced (~29%) in people with schizophrenia. PV expression was reduced in layers III (18%) and IV (31%) with no diagnostic difference in PV mRNA measured by qPCR. FASR expression was increased in schizophrenia (34%). SST expression, but not PV, correlated negatively with FASR and TNFSF13 expression and with interstitial white matter neuron density. To conclude, the current study demonstrates that the magnitude of deficit is greater for SST compared to PV inhibitory interneuron mRNAs in the

OFC in people with schizophrenia. We also demonstrate that reduction in SST, but not PV, interneuron mRNA is linked to increased death receptor signalling in schizophrenia. We suggest that SST interneurons are more vulnerable to increased death receptor signalling than PV interneurons, and that SST interneurons predominantly contribute to the inhibitory interneuron pathology in the OFC in people with schizophrenia.

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Poster

707. Developmental Animal Models of Schizophrenia

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Title: Chronic administration of the neurotrophic agent Cerebrolysin attenuates neuronal abnormalities in the signaling regulation in prefrontal cortex, dorsal hippocampus and nucleus accumbens induced by neonatal ventral hippocampus lesion

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Abstract: Rats with a bilateral neonatal ventral hippocampal lesion (nVHL) are a widely used heuristic neurodevelopmental animal model for studying schizophrenia and have been reported to mimic many schizophrenia-like behaviors. Multiple neurochemical, molecular, and morphological changes have been reported in these rats, including low levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) and atrophy of prefrontal cortex (PFC) pyramidal neurons and medium spiny neurons of the nucleus accumbens (NAcc). All of these changes suggest that a deficit in neurotrophic factors may participate in the development of functional alterations between hippocampus and PFC, interconnecting neural circuits implicated in several aspects of memory and cognition. Recently, we reported that Cerebrolysin may have beneficial effects in the management of the schizophrenia-like behaviors exhibited by nVHL

rats. Cerebrolysin (CBL) is composed of small peptides with activity similar to neurotrophic factors. The neuroprotective effects of CBL may involve multiple mechanisms, including signaling regulation and expression of NGF and BDNF. This study aimed to determine first whether nVH-lesioned rats also exhibited changes in the expression of cell markers for signaling regulation like microtubule-associated protein 2 (MAP-2) and synaptophysin (SYN38) and second whether CBL was capable of reducing neuronal alterations in nVHL rats. Our results suggest that nVH-lesion rats shown a reduction in the expression of MAP2 and a decrease in levels of SYN38 in PFC, NAcc and dorsal hippocampus (DH). Interestingly, CBL treatment ameliorated these changes and increased the expression of MAP-2 and SYN38 in the limbic regions of the nVHL rats. In conclusion, this study demonstrates that CBL promotes a signaling regulation in the PFC, NAcc and DH in the nVHL rats. These findings support the possibility that CBL may have beneficial effects in the management of schizophrenia symptoms. (Supported by: VIEP-BUAP grant (No. FLAG/IND14) and CONACYT grant (No. 138663) to G Flores and NIH grant (AG18440) to E Masliah)

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Poster

707. Developmental Animal Models of Schizophrenia

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Title: Long term effects of late prenatal immune activation: Transcriptome analysis and relevance for psychiatric disorders

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Abstract: Objective: Prenatal maternal infection is an environmental risk factor for several neurodevelopmental disorders, such as schizophrenia and autism. Modelling this epidemiological link in animals shows that prenatal immune challenge is capable of inducing long-term deficits in numerous behavioral and cognitive domains, some of which are highly implicated in schizophrenia and related disorders. In particular, we have shown that exposure to late prenatal infection leads to marked impairments in working memory, social approach and social recognition. The molecular mechanisms underlying such deficits, however, and in particular their relationship with prenatal infection, still remain elusive. To gain more insights into these issues, and possibly discover new potential therapeutic targets, we performed whole genome microarray analysis of the prefrontal cortex of male offspring exposed to in-utero immune challenge. Methods: C57BL/6 mice were treated with the synthetic viral mimetic poly(I:C) (5 mg/kg, i.v.) or control (saline, i.v.) solution on gestation day 17. Offspring were subjected to cognitive and behavioral testing in adulthood. After the behavioral testing, microarray analysis and subsequent q-PCR validation were performed on the prefrontal cortex. Results: Prenatal Poly(I:C) exposure produced significant behavioral and cognitive deficits associated with dysregulation of 185 genes involved in different functional categories, such as nervous system development and function, cellular development and cellular growth and proliferation. Among others, we observed decreased expression of different genes involved in myelination processes, such as MAG (myelin associated glycoprotein), MOG (myelin oligodendrocyte glycoprotein), MOBP (myelin-associated oligodendrocyte basic protein) and MAL (myelin and lymphocyte protein). Conclusions: Our results provided new insight into the molecular mechanisms underlying the relationship between prenatal infection and adult vulnerability to psychiatric disorders, and uncover possible targets for future studies. Indeed, our results point to a possible role of myelination in mediating the detrimental effects of prenatal immune challenge, consistent with similar reports in human studies.

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Poster

707. Developmental Animal Models of Schizophrenia

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Title: Exposure to enriched environment prevents the development of deficit in sensorimotor gating in prenatally MAM treated rats

Authors: *E. BATOR, J. LATUSZ, K. GLOMBIK, K. WEDZONY, M. MACKOWIAK; Inst. of Pharmacol. Polish Acad. of Scienc, Krakow, Poland

Abstract: Schizophrenia is a neurodevelopmental diseases with genetic and environmental background. Although the first symptoms of schizophrenia are seen in adults, the factors cause the risk of this disease might be present in early stage of life. The mechanism of this phenomena is still under investigation, however the adolescent period of life is indicated as a critical for incubation of the psychotic symptoms. Prenatal administration of methylazoxymethanol (MAM) at embryonic day 17 (E17) leads to several behavioural and anatomic abnormalities in adulthood that are similar to those observed in patients with schizophrenia. Moreover, prenatal MAM administration also mimics a dynamic aspect of the illness, since the psychotic symptom, i.e. a deficit in sensorimotor gating process, are present in adult, but not adolescent MAM-treated animals. In the present study we investigated, whether the exposure to enriched environment (EE) during adolescence or early adulthood of postnatal life is able to prevent the impairment in sensorimotor gating process observed in adult prenatally MAM-treated animals. Rat pregnant females (Wistar Han) were injected ip with 22 mg/kg MAM or saline at E17. The offspring were weaned 21 days after birth and only males were used in experiments. Animals were housed in an enriched environment (EE) or standard environment (SE) 24 hours for 7 days in either early adolescence (23rd to 29th day) or early adulthood (63rd to 69th day) before manifestation of psychotic symptoms evoked by prenatal MAM administration. The efficiency of sensorimotor gating process was measured using prepulse-induced inhibition of acoustic startle response (PPI) in adult rats at postnatal days 70 and 90 (P70 and P90, respectively), when the deficit in sensorimotor gating process was observed in prenatally MAM-treated rats. Exposure to EE in early adolescent prevented the appearance of deficit in sensorimotor gating in MAM-treated group measured at P70 and at P90. Moreover, EE given in early adulthood also inhibited the decrease in PPI in MAM-treated group detected at P70, and this effect of EE was also found at P90. The exposure to EE in either adolescence or adulthood did not affect the sensorimotor gating process in VEH groups. The obtained results suggest that exposure to EE before onset of psychosis might prevent MAM-induced behavior abnormalities such as impairment in sensorimotor gating process.

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Poster

707. Developmental Animal Models of Schizophrenia

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Title: IL-6 induced metabolic changes in developing neurons and astroglia

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Abstract: Maternal immune activation and subsequent interleukin 6 (IL6) induction disrupts normal brain development and predispose to developing autism and schizophrenia. To gain a better understanding of the IL-6 dependent metabolome, we separately assessed the effect IL-6 on developing neurons and astroglia by coupling microfluidic technologies to ultra-performance liquid chromatography-ion mobility-mass spectrometry (UPLC-IM-MS). Our results revealed that 1) the use this technology, due to its superb media volume: cell volume ratio, is ideally suited for analysis of cell-type specific exo-metabolome signatures; 2) developing neurons have low secretory activity at baseline, while astroglia show strong metabolic activity; 3) both neurons and astroglia respond to IL-6 exposure in a cell-type specific fashion; 4) astroglial response to IL-6 stimulation is predominantly characterized by increased levels of analytes, while neurons mostly depress their metabolic activity; and 5) disturbances in glycerophospholipid metabolism and trypophane/kynurenine metabolite secretion are two important mechanisms by which IL-6 affects the developing nervous system. Our findings are critical for understanding the mechanism by which IL-6 disrupts brain function, and provides insights into the cascade of

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Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Autoradiographic studies of maternal immune activation in male and female offspring

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Abstract: A major risk factor for schizophrenia (SCZ) is maternal infection during pregnancy, and extensive animal research seeks to explore the long-term neural and behavioral effects of immune-mediated disruption of early brain development. In the prenatal polyriboinosinic–polyribocytidilic acid (poly-I:C) animal model of SCZ, a single injection of pregnant rats with the viral mimic poly-I:C (4 mg/Kg IV on gestational day 15), induced a wide spectrum of SCZ-relevant behavioral and structural brain abnormalities in adult male and female offspring. These abnormalities were shown to be preventable by peripubertal treatment with risperidone (0.045 mg/Kg/day on postnatal days 34-47) and other antipsychotic drugs. The present study examined the effects of prenatal poly-I:C and peripubertal risperidone on hippocampal neuroinflammation and 5HT2a receptors in adult male and female offspring. Coronal brain sections from 5 animals/sex/treatment were incubated with [^{3}H]PK11195 and [^{3}H]ketanserine respectively, and films analyzed using quantitative autoradiography. In male offspring, prenatal poly-I:C was associated with significant elevations in specific binding of [^{3}H]PK11195 in the dorsal hippocampus, which were prevented by risperidone. Risperidone pretreatment also reduced 5HT2a receptor density in poly-I:C exposed male rats. A more restricted neuroinflammatory response was observed in ventral hippocampus of female offspring, and this increase, too, was prevented by risperidone. However, female offspring did not show any changes in 5HT2a density in hippocampus due to either prenatal poly-I:C or peripubertal risperidone. These findings complement previously reported sex differences in brain structural and behavioral trajectories following prenatal poly-I:C, whereby female offspring were less affected than male

offspring, and resonate with the clinical picture of later onset and milder disease course of SCZ in women. The findings also support a role for neuroinflammation in hippocampus in the deleterious effects of prenatal poly-I:C administration as well as an anti-inflammatory effect of peripubertal risperidone in this model, which may be relevant to prevention of SCZ.

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Poster

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CNPq

Title: Role of CB1 receptors in the Contextual Fear Conditioning (CFC) deficit in an animal model of schizophrenia - the Spontaneously Hypertensive Rats (SHR)

Authors: ***F. F. PERES**[1], R. LEVIN[2], C. M. SANTOS[2], V. ALMEIDA[2], J. E. HALLAK[3], A. W. ZUARDI[3], J. A. CRIPPA[3], V. C. ABÍLIO[2];
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Abstract: Clinical findings suggest that the cannabinoids and the endocannabinoid system may be implicated in the pathophysiology and treatment of schizophrenia. Post-mortem studies show that schizophrenia patients present an increased density of the cannabinoid CB1 receptor binding in brain regions involved in this disorder. The Spontaneously Hypertensive Rat (SHR) strain presents a schizophrenia behavioral phenotype that is specifically attenuated by antipsychotic drugs and potentiated by proschizophrenia manipulations. Based on these findings, we have suggested this strain as an animal model to study several aspects of schizophrenia. The aim of this study was to evaluate the effects of WIN55212,2 (WIN - CB1 agonist) or rimonabant (RIMO - CB1 antagonist) on the contextual fear conditioning (CFC) deficit presented by this strain. Moreover, we also quantified the density of CB1 receptors in brain areas associated with the pathophysiology of schizophrenia on the SHR strain. Male three-months-old WR and SHR were used. For the behavioral experiments, WR and SHR were treated with vehicle, 0.1, 0.3, 1 or 3 mg/kg WIN (experiment 1) or vehicle, 0.75, 1.5 or 3 mg/kg RIMO (experiment 2). Thirty minutes later, the animals were submitted to the acquisition session of the CFC in which 0.4 mA

footshocks were presented in association with a context. During the test session, performed 24h later, the freezing duration (a fear response) was quantified in the absence of footshocks. CB1 density was measured by immunohistochemistry on several brain areas. For the CFC, the SHRs treated with vehicle presented decreased freezing response when compared to WRs. The administration of 3 mg/kg rimonabant or 0.1 mg/kg WIN reversed this impairment, while the treatment with 1 and 3 mg/kg WIN worsened it. On WRs, 0.3 mg/kg WIN administration increased freezing response while 3 mg/kg WIN decreased it and rimonabant was not able to modify it on any of the doses used. In addition, the SHR strain presented increased density of CB1 on the hippocampus sub-region CA3 as well as on pre-limbic, infra-limbic and anterior cingulated cortices when compared to WRs. Our data revealed increased CB1 receptors density on brain areas relevant to the pathophysiology of schizophrenia. These results are in accordance with clinical findings that demonstrate an increase in CB1 density on corticolimbic regions of schizophrenia patients and therefore reinforce the suggestion of the SHR strain as an animal model to study several aspects of schizophrenia. Finally, the ability of WIN and rimonabant in modifying the CFC deficits of SHRs points to the endocannabinoid system as a potential therapeutic target to treat schizophrenia.

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Poster

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Mitsubishi-Tanabe

Title: The systemically active kynurenine aminotransferase II inhibitor BFF816 attenuates contextual memory deficit induced by chronic prenatal kynurenine elevation in rats

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Abstract: Schizophrenia (SZ), a catastrophic psychiatric disorder, results from a combination of genetic and environmental factors. KYNA is an endogenous antagonist of α7 nicotinic acetylcholine (α7nACh) and NMDA receptors, and increases in brain KYNA have been implicated in the pathology of SZ. Based on the neurodevelopmental hypothesis of SZ etiology, we recently developed a naturalistic model to study the KYNA hypothesis of SZ by adding the KYNA precursor kynurenine (kyn) (100 mg/day) to the chow fed to pregnant dams from embryonic day (ED) 15 to ED 22 (control: ECon; kyn-treated: EKyn). Upon termination of the treatment, all rats were fed normal rodent chow until the animals were evaluated in adulthood, i.e. on postnatal days (PD) 56-80. Unexpectedly, tissue KYNA levels remained increased in the hippocampus of adult EKyn animals, and prenatal kyn treatment was found to cause significant hippocampus-dependent cognitive dysfunctions in adulthood (Pocivavsek et al., Psychopharmacology, 2014). We now examined whether BFF816 a systemically active inhibitor of kynurenine aminotransferase II (KAT II), a major KYNA-synthesizing enzyme in the brain (Wu et al., Schiz. Bull., 2014), would affect neurochemistry and behavior in adult EKyn animals. Offspring were cross-fostered, i.e. ECon and EKyn pups were equally mixed within a nursing litter. Determined by microdialysis in the hippocampus of unanesthetized adult rats, basal extracellular KYNA levels were modestly but significantly elevated in EKyn rats (ECon: 2.3 ± 0.1 nM; EKyn: 2.8 ± 0.1 nM; n = 7-8 per group), and this effect was paralleled by a significant decrease in extracellular glutamate (ECon: 1.9 ± 0.03 μM; EKyn 1.6 ± 0.02 μM; n = 4-5 per group). BFF816 (30 mg/kg), administered p.o. after the collection of 4 baseline measurements, reduced KYNA levels in both ECon and EKyn adult rats, reverting the KYNA levels in EKyn animals to the ECon baseline. BFF816 also raised extracellular glutamate levels in both ECon and EKyn adult rats (189% and 169% respectively). In separate adult animals, we tested contextual memory using the passive avoidance paradigm (PAP). Prenatal kyn treatment caused significant PAP deficits, evidenced as decreased avoidance latency during the retention trial (ECon: 118 ± 20 s; EKyn: 43 ± 13 s). Pretreatment with BFF816 (30 mg/kg, p.o.) 5 min prior to acquisition testing on day 1 attenuated this memory-impairment (ECon: 100 ± 21 s; EKyn: 116 ± 23 s; n = 14-17 per group). Collectively, our results indicate that acute inhibition of KAT II in adulthood may be sufficient to overcome contextual memory deficits induced by elevated brain KYNA during a vulnerable period in early brain development.

Disclosures: A. Pocivavsek: None. **H. Wu:** None. **M. Okuyama:** None. **Y. Kajii:** None. **G.I. Elmer:** None. **J.P. Bruno:** None. **R. Schwarcz:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 707.08/V29

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Commonwealth Health research Board of Virginia Award 34-10 to JKS, JHP, JJW, KFS

Title: Examining the motoric effects of vesicular monoamine transporter 1 depletion (VMAT1-/-) in young and aged mice

Authors: *C. R. MERRITT[1], K. A. WEBSTER[1], J. J. WINDLE[2], J. K. STEWART[3], J. H. PORTER[1];
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Abstract: Vesicular monoamine transporter 1 (VMAT1) is a protein, encoded by the gene SCL18A1, responsible for moving monoamines from the cytosol to the vesicle after reuptake of the monoamines has occurred. It has been proposed that serotonin is important in embryonic development, and VMAT1 mRNA is expressed in many more places during this period. Deletion of VMAT1 could lead to improper or suboptimal usage of serotonin during development which may lead to serious deficits in cognitive as well as muscular development. These developmental deficits could affect weight, strength, and could impact other aspects of the animal's behavior. To examine the effects of VMAT1 deletion on muscle and motor development locomotor activity, accelerating rotarod, and grip strength were tested in both VMAT1-/- and VMAT1-/- (littermate controls). We also examined young and aged mice to investigate the potential long term effects of the VMAT1 knockout. Mice were generated and were separated at 8 weeks and singly housed. Mice were tested at 4-5 months for young mice and at 12-15 months for aged mice. For locomotor activity mice were placed in the middle of the locomotor activity box and allowed to freely explore for one hour and the mice were tested for 6 consecutive days to examine potential differences in habituation. For the accelerating rotarod the mice were placed on the rotarod at a speed of 1 rotation/minute. The speed of the rotarod, increasing 1 RPM every 3 seconds until the mouse lost its grip and fell, or stopped making forward movement. Both the latency to fall and the speed at which the animal fell were recorded. Mice were tested for 10 consecutive trials for 3 days with a minimum of 30 seconds between trials and one day between each test day. In the grip strength task mice were held at the base of the tail and allowed to grab a metal triangle that was attached to a force meter. The mice were then slowly pulled back away from the meter at a constant rate, until they could no longer maintain grip with both paws. Mice were tested for 3 consecutive trials for 3 days with a day between each test day. Results from this

study revealed that there were no significant differences between VMAT1 -/- and VMAT1+/+ mice in any of the motoric measures in either of the young or aged cohorts. These data suggest that behavioral and cognitive deficits seen in VMAT1 -/- mice relative to VMAT1+/+ mice are not due to deficits in strength or motor coordination.

Disclosures: C.R. Merritt: None. **K.A. Webster:** None. **J.J. Windle:** None. **J.K. Stewart:** None. **J.H. Porter:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

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Program#/Poster#: 707.09/V30

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Stanley Medical Research Institute

NIH Silvio O. Conte Center Grant PAR-08 194

Title: Immune response to Toxoplasma can be responsible for some neurobehavioral changes in chronically infected mice

Authors: *G. KANNAN[1], K. L. GRESSITT[2], J. A. CRAWFORD[3], L. JONES-BRANDO[2], R. H. YOLKEN[2], M. V. PLETNIKOV[3];
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Abstract: The protozoan parasite Toxoplasma gondii (T. gondii) has been shown to affect behavior. Modulation of host behavior has long been thought to be due to parasite cysts in the brain. In order to test this hypothesis, we compared the behavioral effects of live, cyst-forming T. gondii and UV-inactivated parasites that are unable to replicate and form cysts. We show that mice administered live and inactivated T. gondii exhibit comparable responses to the NMDAR antagonist MK-801in pre-pulse inhibition of the acoustic startle and cue-dependent fear conditioning. In contrast, in the dark-light box test, live parasites produced increased anxiety while inactivated T. gondii led to decreased anxiety in mice. These findings provide evidence that the immune response to T. gondii contributes to chronic T. gondii infection-produced behavioral alterations.

Disclosures: G. Kannan: None. **K.L. Gressitt:** None. **J.A. Crawford:** None. **L. Jones-Brando:** None. **R.H. Yolken:** None. **M.V. Pletnikov:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 707.10/V31

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Commonwealth Health Research Board of Virginia Award 34-10 to JKS, JHP, KFS

Title: Age and sex differences in operant learning and corticosterone in vesicular monoamine transporter 1 depleted mice (VMAT1-/-)

Authors: *J. K. STEWART[1], K. A. WEBSTER[2], K. FISCHER-STENGER[4], Y. GENG[4], E. C. GONYE[4], J. J. WINDLE[3], J. H. PORTER[2];
[1]Biol., [2]Psychology, [3]Human and Mol. Genet., VA Commonwealth Univ., RICHMOND, VA;
[4]Biol., Univ. of Richmond, Richmond, VA

Abstract: Vesicular monoamine transporter 1 (VMAT1) in humans and rodents is the primary vesicular transporter in adrenal medulla, but the VMAT1 gene also is expressed in specific areas of brain. Association of the VMAT1 gene SLC18A1 with psychiatric disorders, particularly in females, led to investigation of behavioral differences in VMAT1-/- and VMAT1+/+ (littermate control) mice. Previously, we reported delayed learning in VMAT1-/- mice at age 4-5 months as compared to VMAT1+/+ mice of the same age. We now report a significantly greater delay in learning in female than male VMAT1-/- mice at age 4-5 months. Operant learning was assessed in an autoshaping task, in which acquisition of a conditioned lever-press response for food reward was measured under mild deprivation conditions (food reduced to maintain 85% body weight). As expected in C57BL/6 mice, corticosterone fecal metabolites (an index of mouse plasma corticosterone) were higher in young females than in young males, but corticosterone metabolites also were higher in young female VMAT1-/- mice than in young VMAT1+/+ female mice. No significant differences in corticosterone were observed in male VMAT1-/- and VMAT1+/+ mice. Thus, there appeared to be a correlation between the behavioral and corticosterone differences in the young female mice. In aged mice (12-15 months of age), learning differences were no longer evident between VMAT1-/- and VMAT1+/+ animals. Although corticosterone metabolites were still higher in the aged female mice than in the aged males, there were no significant differences in corticosterone in VMAT1-/- and VMAT1+/+ mice in either sex. Immunohistochemical data confirmed that there was a VMAT1 deficiency in the adrenals of both young and aged VMAT1-/- mice as compared to VMAT1+/+ mice. These

findings are interesting in view of the more prevalent association of VMAT1 with psychiatric disorders in females and the well documented corticosteroid enhancement of behavioral disorders.

Disclosures: **J.K. Stewart:** None. **K.A. Webster:** None. **K. Fischer-Stenger:** None. **J.J. Windle:** None. **J.H. Porter:** None. **Y. Geng:** None. **E.C. Gonye:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Commonwealth Health research Board of Virginia Award 34-10 to JKS, JHP, JJW, KFS

Title: Exploring behavioral outcomes of vesicular monoamine transporter 1 depletion (VMAT1-/-) in young and aged mice

Authors: ***K. A. WEBSTER**[1], P. M. BEARDSLEY[2], J. J. WINDLE[3], J. K. STEWART[4], J. H. PORTER[1];
[1]Psychology, [2]Pharmacol. & Toxicology, [3]Human Genet., [4]Biol., Virginia Commonwealth Univ., Richmond, VA

Abstract: Interest in the role of the vesicular monoamine transporter 1 (VMAT1) gene (SLC18A1) in psychiatric disorders increased after discovery that VMAT1 is expressed in both human and mouse brain and that genetic variants of VMAT1 have been reported in schizophrenic and bipolar I disorder patients. In order to investigate potential behavioral effects caused by depletion of VMAT1 and to explore how these deficits may change in aged animals, we tested both young and aged VMAT1-/- and VMAT1+/+ mice in the Morris water maze and in the progressive ratio task. We also tested pre-pulse inhibition (PPI) in young mice. Progressive ratio is an operant task in which the number of lever presses required for each successive food reward is increased in an exponential fashion. The session lasted for 60 min or until 300 sec elapsed since the last lever press. The main dependent measure is breakpoint, which is the last completed ratio. The progressive ratio task is used to model deficits in motivational behavior that is seen in schizophrenia and in the depressive stages of bipolar disorder. The Morris water maze tests a mouse's ability to locate and remember the location of a submerged platform, using only

spatial cues. Mice were tested 4 times a day over 5 days with the same hidden platform position. On the 6th day, the location of the platform was moved and a signal (black film canister) was placed on top of the platform. The main dependent measures were latency to platform (sec) and swim speed (cm/sec). In pre-pulse inhibition mice were exposed to 13 blocks containing 7 trials arranged in a pseudorandom order. Mice were exposed to a 120 dB startle tone that was preceded by 69-85 dB pre-pulse tones. The test measures how much the animal startles (jumps) to the 120 dB startle tone, and how much the varying dB levels of the pre-pulse tone inhibit the startle response. Pre-pulse inhibition is an endophenotype for schizophrenia, characterized by an inability of the pre-pulse to reduce the startle reaction to the pulse tone. Results from the present study found that there were no differences in latency to platform or swim speed between young or aged VMAT1 -/- and VMAT1 +/+ mice. In progressive ratio VMAT1 -/- mice were not significantly different from VAMT1 +/+ mice in either young or aged cohorts. In pre-pulse inhibition the VMAT1 -/- mice had a significant deficit in the ability to inhibit startle reactivity relative to VMAT1 +/+ mice. Taken together these results suggest that dysfunction of VMAT1 plays a role in sensorimotor gating deficits, a common symptom in schizophrenic patients. However, there were no significant deficits in spatial memory or in motivation-like behavior.

Disclosures: K.A. Webster: None. **P.M. Beardsley:** None. **J.J. Windle:** None. **J.K. Stewart:** None. **J.H. Porter:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

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Program#/Poster#: 707.12/W1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH091407

NIH Grant MH094670

HHMI

Title: Delayed cortical maturation, enhanced fear conditioning and social deficits in mice exposed to perinatal ketamine

Authors: *M. BEHRENS[1], A. KHAN[2], N. D. JOHNSON[1], E. A. MUKAMEL[3], J. D. LUCERO[4], T. J. SEJNOWSKI[5], V. B. RISBROUGH[2], S. B. POWELL[2];

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Abstract: There is increasing evidence that disruptions in cortical inhibitory networks contribute to negative symptoms and anxiety associated with schizophrenia. These symptoms are extremely debilitating and remain under-treated with current antipsychotic medications. GABAergic interneurons are dysfunctional in a number of brain regions in schizophrenia, including the frontal cortex and hippocampus. Converging evidence from developmental animal models indicates that parvalbumin-positive (PV+) interneurons are particularly critical to disrupted behavior in these models. PV+ fast spiking interneurons modulate the activity of pyramidal neurons and are a key regulatory of the excitatory-inhibitory balance in cortex. We have previously shown that perinatal exposure of mice to the NMDA receptor antagonist ketamine (pNK) results in loss of PV+ immunoreactivity in the prelimbic cortex, in decreased inhibitory drive from fast-spiking interneurons. We have also shown that pNK produces long term alterations in transcriptional activity suggestive of delayed maturation. Among the differentially expressed genes, we have found several schizophrenia-related genes such as Reln, Erbb4, Grin2a, Grin2b, or Cit, some of which are expressed preferentially in GABAergic neurons. To further test for delayed development we analyzed whether other maturational features of PV+ neurons showed long term alterations. We have found that perineuronal nets, which are dense extracellular matrix structures that surround the soma and dendrites of many neurons in the mature CNS, in particular PV+ interneurons, are reduced in pNK animals. Furthermore, ketamine administration during the neonatal period produced enduring behavioral deficits. Mice exposed to ketamine during the perinatal period showed decreased social approach behavior and enhanced contextual and cued fear conditioning compared to saline-injected mice. These data indicate that the loss of PV+ interneurons associated with perinatal ketamine administration leads to sustained disinhibition of prefrontal cortex, to enhanced fear expression, and social deficits in mice. Increased fear learning is consistent with the hypothesis that cortical disinhibition in pNK mice leads to increased excitatory output from prelimbic cortex (PL) to the basolateral amygdala (BLA). This circuit has been shown to be important for the fear learning and emotional memory.

Disclosures: **M. Behrens:** None. **A. Khan:** None. **N.D. Johnson:** None. **E.A. Mukamel:** None. **J.D. Lucero:** None. **T.J. Sejnowski:** None. **V.B. Risbrough:** None. **S.B. Powell:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

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Program#/Poster#: 707.13/W2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Lundbeck foundation, Denmark

Title: Zinc finger protein 804A (ZNF804A) levels in the developing brain

Authors: *E. BENEDIKZ, K. RICH, K. HVID;
Inst. of Mol. Medicine, Neurobio. Res., Univ. of Southern Denmark, Odense, Denmark

Abstract: Schizophrenia is a severe psychiatric disorder with lifetime prevalence of about 1%. The disease is characterized by delusions, hallucinations, altered cognition, emotional reactivity and disorganized behavior. Research increasingly suggests that schizophrenia is a subtle disorder of brain development and plasticity. However, despite extensive research, the etiology and pathophysiology remain poorly understood. Genetics play an important role in the disease and heritability is around 80%. Recently ZNF804A was the first gene to achieve genome-wide significance for psychosis and several genome-wide association studies have since confirmed an association between schizophrenia and ZNF804A. The function of ZNF804A and its role in the disease are unknown. Interestingly the schizophrenia susceptibility genotype of ZNF804A is associated with altered connectivity in the dorsolateral prefrontal cortex, the hippocampus, and the amygdala. Altered connectivity within and between these brain regions has been associated with schizophrenia. In this study we have analyzed the mRNA levels of ZNF804A in different brain regions and at different ages in rats using qPCR. We have also invstigated human embryonic brain tissue from 5,5 to 10,5 week embryonic brain tissue. Our results show that expression of ZNF804A is developmentally regulated and increases significantly in the brain of embryonic day 18. In cortex and cerebellum the mRNA levels of ZNF804A are high around birth and then decrease to the adult level. The expression of ZNF804A is also developmentally regulated in the hippocampus, but after decreasing from the postnatal levels, the expression increases again in the adult. Interestingly the expression is much higher in CA1 than in CA3 or the dentate gyrus. In the human embryonic tissue the levels are much lower than that seen in the rat tissue from E16 and older, suggesting that the human tissue is developemntally much younger. These results suggest that ZNF804A plays a role in neuronal development.

Disclosures: **E. Benedikz:** None. **K. Rich:** None. **K. Hvid:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 707.14/W3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: USPHS MH57440

NIH T32 DA031111

Title: Altered NADPH-diaphorase activity in the prefrontal cortex in a neurodevelopmental disruption model of schizophrenia

Authors: *S. M. DIBBLE, A. A. GRACE;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Hypofunctionality of the prefrontal cortex as well as abnormalities in cortical interneurons are observed in schizophrenia, and have been hypothesized to underlie cognitive impairments in this disorder. Nitric oxide, a gaseous neuromodulator that facilitates plasticity and dopaminergic signaling, is produced by a sparse subclass of interneurons via the enzyme neuronal nitric oxide synthase (nNOS). Heteroplasia of nNOS+ interneurons has been observed in postmortem tissue; specifically, it has been hypothesized nNOS+ interneurons fail to migrate into the neocortical layers in schizophrenia. Here, we examine nNOS activity in the prefrontal cortices of a rodent neurodevelopmental disruption model of schizophrenia, generated by prenatal administration of the antiproliferative agent methylazoxymethanol (MAM) on embryonic day 17. Our preliminary results suggest a reduction in NADPH-diaphorase activity in the prefrontal cortex of adult MAM-17 rats as compared to saline controls. A reduction in nitrergic signaling may contribute to the cognitive impairments, such as behavioral inflexibility observed in the MAM-17 rats, as well as the reduction in NREM sleep reported by others in these rats. Whether the reduction in NADPH-diaphorase activity reflects a decrease in the number of cortical nNOS+ interneurons in the MAM-17 model or a decrease in nNOS activity secondary to attenuated cortical activity remains to be explored.

Disclosures: S.M. Dibble: None. **A.A. Grace:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 707.15/W4

Topic: A.10. Adolescent Development

Support: NIH Grant R01-MH086507

Title: Early adolescent NMDA receptor blockade impairs the maturation of the prefrontal GABAergic system and PFC-dependent fear extinction behavior

Authors: *D. R. THOMASES, E. FLORES-BARRERA, J. A. ROSENKRANZ, K. Y. TSENG; Cell. and Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: While many brain regions are fully mature by the time of adolescence, the prefrontal cortex (PFC) continues to undergo many structural and functional changes during this developmental period. We have recently found that a transient blockade of NMDA receptors during early adolescence disrupts the developmental regulation of PFC network inhibition, measured in adulthood. These findings suggest that a developmental impairment of GABAergic transmission in the PFC likely underlies such deficits. In addition, many PFC-dependent behaviors also require proper levels of prefrontal GABAergic function, pointing towards concurrent behavioral deficits associated with impaired maturation of PFC network inhibition. To address these issues, we determined how GABAergic transmission in the PFC and the extinction of trace fear memory become altered in adult rats that received repeated injections of the non-competitive NMDA receptor antagonist MK-801 during early adolescence. Typically GABAergic transmission onto layer V pyramidal neurons increases sharply from adolescence to adulthood. However, this increase is attenuated following early adolescent MK-801 exposure. Notably, bath application of the CB1R agonist WIN 55,212-2 reduced GABAergic transmission similarly in both MK-801- and saline-treated rats. Given that CB1R activation reduces GABAergic output specifically from non-fast-spiking interneurons, the results point towards a mechanism of reduced fast-spiking interneuron function underlying the GABAergic deficits produced by the early adolescent MK-801 treatment. At the behavioral level, we also found that the extinction of learned fear memory is markedly reduced in the MK-801-treated group. While control rats exhibited conditioned freezing to the tone that diminished over repeated trial, MK-801-treated rats showed a significantly slower attenuation of conditioning freezing over trials, indicating that the acquisition of fear extinction is impaired. Together, these data indicate that a transient early adolescent NMDAR blockade is sufficient to produce an enduring attenuation of GABAergic transmission in the PFC associated with reduced PFC-dependent behavior in adulthood. Such deficits are likely due to a developmental impairment of PFC GABAergic maturation of fast-spiking interneurons as these prefrontal disruptions were lacking in the adult-treated group.

Disclosures: D.R. Thomases: None. **E. Flores-Barrera:** None. **J.A. Rosenkranz:** None. **K.Y. Tseng:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH57440

NIH Grant DA 15408

Title: Adolescent-specific abnormal stress responsivity in a rodent developmental disruption model of schizophrenia

Authors: *E. C. ZIMMERMAN[1], A. A. GRACE[2];
[1]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Neuroscience, Psychiatry and Psychology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Numerous studies implicate adolescent stress in the pathophysiology of schizophrenia. It has been hypothesized that stress exposure in adolescence could be exacerbated by a predisposition towards increased stress sensitivity, leading to prodromal symptoms and eventually psychosis. Rats born to dams administered with the DNA methylating agent methylaxozymethanol acetate (MAM) at gestational day (GD) 17 exhibit behavioral and anatomical abnormalities analogous to those observed in patients with schizophrenia. We reported previously that diazepam administration to control stress responses in the peripubertal period, postnatal day (PND) 31-40, prevents the emergence of hyperdopaminergic phenotypes observed in adult MAM-treated animals. We hypothesized that peripubertal MAM animals would exhibit altered hypothalamic-pituitary-adrenal (HPA) axis responses to both acute and repeated stressors as compared to their saline-treated counterparts (SAL). Adolescent MAM animals were exposed to 10 days of inescapable footshock stress in the peripubertal period (PND 31-40). Plasma corticosterone levels were measured following acute and repeated footshock exposure. In addition, in a separate cohort of adult MAM rats plasma corticosterone levels were measured just before and immediately after (<5min) a single session of footshock. Finally, metyrapone, an inhibitor of adrenal 11-B-hydroxylase, was administered to MAM rats once per day for 10 days from PND 31-40, and amphetamine-induced hyperlocomotion was measured in adulthood. In the peripubertal period, MAM rats showed a disruption in HPA axis responses to stress, exhibiting attenuated corticosterone levels after acute footshock that did not adapt to 10 days of repeated stress exposure. In contrast, corticosterone release in response to acute footshock stress in adult MAM animals mirrored that of SAL counterparts. Preliminary findings suggest that metyrapone had opposite effects in MAM and SAL animals: amphetamine-induced hyperlocomotion was exacerbated in MAM animals and attenuated in SAL animals. Taken

together, our findings suggest that peripubertal MAM animals are unable to integrate appropriate responses to acute stress and unable to adapt to repeated stress over time, and that manipulating HPA axis signaling in the peripubertal period modulates dopamine hyperresponsivity observed in adult MAM animals. In addition, these findings support the idea that stress during puberty and adolescence could be a contributing factor to the transition to psychosis, and that controlling stress at this vulnerable period may and prevent the emergence of psychosis later in life.

Disclosures: E.C. Zimmerman: None. **A.A. Grace:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH097997

Title: A master regulator of hindbrain development, EGR2 is upregulated in the forebrain during prenatal stages of development in DISC1 mutant mice

Authors: *P. L. KATSEL[1], W. TAN[1], P. FAM[1], B. ABAZYAN[2], M. XIA[2], M. PLETNIKOV[2], V. HAROUTUNIAN[1,3];
[1]Dept Psych, Mount Sinai Med. Ctr., New York, NY; [2]The Johns Hopkins Univ., Baltimore, MD; [3]JJ Peters VA Med. Ctr., Bronx, NY

Abstract: Strong evidence corroborates involvement of oligodendrocyte (OLG) dysfunction in the pathophysiology of schizophrenia (SZ). DISC1 is a risk gene for several major mental illnesses including SZ. We previously showed that the forebrain-restricted expression of mutant human DISC1 (hDISC1) exerts a significant influence on oligodendrogenesis during early development and in adult hDISC1 mice, evidenced by premature OLG differentiation and increased proliferation of their progenitors in forebrain regions. Concurrent reduction of OLG progenitor markers in hindbrain regions during fetal stage suggested expansion of hindbrain glial progenitors into the forebrain of hDISC1 mice. We tested this hypothesis by examining gene and protein expression of the molecular determinants of hindbrain OLG development (EGR2/Krox20 and Nkx2-2) in samples from forebrain and hindbrain regions at E15, P0, P14 and P21 days of hDISC1 mice. We found forebrain-restricted upregulation of gene and protein of the hindbrain markers of OLG progenitors (EGR2 and Nkx2-2) at E15, coinciding with the peaks of

endogenous and human mutant DISC1 expression. This increased fetal expression of EGR2 was followed by its down-regulation in forebrain during CNS myelination from P14 to P21. In addition, we detected increased expression of early OLG progenitor markers (PDGFRA and NG2) and myelinating OLG markers (CNP and MAG) during prenatal but not postnatal periods. Levels of OLG progenitors, however, remained increased in frontal cortex and cerebellum during the peak of myelination at P14 in hDISC1 mice compared to controls. Our findings show a significant effect of hDISC1 on hindbrain OLG development and suggest expansion of OLG progenitors responsible for developmental positioning of OLG identity cells along the rostrocaudal axis. Dislocation of OLG lineage cells as a result of their abnormal migration and premature differentiation may affect cortical organization of the brain. Given the critical role of DISC1 in migration of neuronal and glial progenitors during brain development, our results provide new clues for the developmental mechanisms contributing to oligodendrocyte dysfunction in SZ.

Disclosures: **P.L. Katsel:** None. **W. Tan:** None. **P. Fam:** None. **B. Abazyan:** None. **M. Xia:** None. **M. Pletnikov:** None. **V. Haroutunian:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH57440

Title: Role of the basolateral amygdala in impaired context processing in the MAM model of schizophrenia

Authors: *K. M. GILL, A. A. GRACE;
Dept. of Neurosci., Univ. Pittsburgh, Pittsburgh, PA

Abstract: Schizophrenia (SZ) patients exhibit morphological reductions and alterations in the extracellular matrix in the post-mortem basolateral amygdala (BLA), and also show aberrant amygdala activation prior to disease onset in at-risk populations. Altered connectivity between BLA and the ventral hippocampus (vHPC) is of particular consequence in SZ given the considerable evidence substantiating the involvement of the vHPC in generating the abnormal dopamine system activity and cognitive dysfunction. In the present study, we examined

contextual fear learning in the methylazoxymethanol acetate (MAM) developmental model of SZ in the rodent. In particular, we tested the role of the BLA in MAM animals during context pre-exposure in the immediate shock fear learning paradigm. Saline and MAM-treated offspring were bilaterally implanted with infusion guide cannula above the BLA one week prior to behavioral testing. Animals were subsequently trained in a modified context pre-exposure fear conditioning paradigm involving two distinct conditioning contexts (Contexts A and B). Animals were infused with either dPBS or muscimol/baclofen prior to pre-exposure to a single context. Animals in the context-shift group were pre-exposed to Context B, while animals in the context-same group were pre-exposed to Context A, the conditioning context. Context pre-exposure took place 24 hours prior to conditioning. For conditioning, all rats received a 0.4-mA, 2-sec shock 120-sec after being placed in Context A. 24-hr after conditioning, animals were placed in the conditioning context (Context A), and freezing scored for 5-min. Saline rats demonstrated the expected increase in freezing behavior when the pre-exposure context was the same as the conditioning context. In contrast, MAM animals exhibited reduced fear responses compared to normal rats. In addition, MAM animals expressed comparable levels of freezing in both the context-shift and context-same groups. This would suggest greater fear generalization in MAM animals that interfered with discrimination between contexts. Preliminary results indicate that BLA inactivation during pre-exposure in MAM rats reduces this fear generalization. Alterations in normal vHPC function in MAM rats interfered with the ability to engage consolidated contextual information attained during context pre-exposure occurring prior to fear conditioning. In addition, reducing excessive BLA output to the vHPC via transient inactivation during context pre-exposure reduced fear generalization in MAM animals. This data provides insight into the pathophysiology of SZ and also its prevention in susceptible individuals.

Disclosures: **K.M. Gill:** None. **A.A. Grace:** None.

Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 707.19/W8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: French Ministery of Research

Fondation pour la recherche medicale

inserm

Fondation Jerome Lejeune

Title: Reelin, an extracellular matrix protein linked to early onset psychiatric diseases, drives postnatal development of the prefrontal cortex

Authors: *B. LAMINE, J. IAFRATI, J. OREJARENA, O. LASSALLE, C. GONZALEZ CAMPO, P. CHAVIS;
INSERM, Marseille, France

Abstract: Reelin is a large secreted glycoprotein present in the brain extracellular matrix. Reelin modulates neuronal development and participates to the functions of adult central synapses. Our past work fueled the concept that in the postnatal brain, reelin is required for the homeostasis of glutamatergic receptors that compose the majority of excitatory synapses. Reelin dysfunction has been proposed to contribute to the etiology of several psychiatric disorders such as schizophrenia, major depression and bipolar disorders. Moreover disruption of the maturation of the PFC functions participates to these psychiatric diseases. However the role of reelin in maturation and plasticity of glutamate connectivity in the prefrontal cortex (PFC) has never been investigated. We combined ex-vivo electrophysiological recordings, 3D imaging and behavioral approaches to study the postnatal maturation of PFC connectivity in the reelin-haploinsufficient heterozygote reeler mouse. Our most recent data show that reelin is essential for successful structural, functional and behavioral development of juvenile prefrontal circuits.

Disclosures: B. Lamine: None. **J. Iafrati:** None. **J. Orejarena:** None. **O. Lassalle:** None. **C. Gonzalez Campo:** None. **P. Chavis:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NSF-REU 0851194

Title: Postnatal injection of homocysteic acid leads to the development of a mixed state of mania and depression-like behavior in female, but not male, Sprague Dawley rats

Authors: *L. A. CHASE[1,2], G. FLORES[3], J. JOHNSON[4], J. GABLE[2], C. C. BARNEY[4]; [1]Hope Col., HOLLAND, MI; [2]Neurosci. Program, Hope Col., Holland, MI; [3]Biochem. and Mol. Biol. Program, Hope Col., HOLLAND, MI; [4]Biol. Dept., Hope Col., Holland, MI

Abstract: Recent evidence suggests that hyperfunction of N-methyl-D-aspartate receptors (NMDA) may be an important factor in the pathophysiology of major depressive disorder (MDD) and bipolar disorder. The NMDA receptor agonist, homocysteic acid (HCA), is an endogenous compound that is formed from the oxidation of homocysteine. Since hyperhomocysteinemia is a risk factor for several neuropsychiatric disorders, including MDD, we sought to test the hypothesis that elevated levels HCA levels in developing rats may lead to changes in NMDA receptor expression and the development of behaviors associated MDD and/or bipolar disorder. To test this hypothesis, we performed daily i.p. injections of either HCA or saline in postnatal male and female rats for 14 days beginning at day P2. Six weeks later, we observed that female, HCA-treated rats displayed increased risk-taking behavior, reduced social behavior, novelty-induced hyperlocomotion, anhedonia, and reduced sensitivity to pain compared to control and male-HCA treated rats. However, there were no changes in paired pulse inhibition, motor coordination or weight gain over the course of the experiment. Consistent with these findings, we observed that HCA exposure led to an increase in the expression of the NMDAR2 subunit and GAD-67 in the cortex and hippocampus of female, but not male, rats. Given that MDD and bipolar disorder are sexually dimorphic and observed more often in females compared to males, these data suggest that early postnatal exposure to HCA may serve as an excellent animal model for these disorders.

Disclosures: L.A. Chase: None. **G. Flores:** None. **J. Johnson:** None. **J. Gable:** None. **C.C. Barney:** None.

Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Capes

FPA

Title: Expression of EAAC1 but not AMPA glutamate receptors is increased in the prefrontal cortex of rats reared in isolation from weaning

Authors: *M. A. FERREIRA[1], G. BORGES[1], J. RODRIGUES[1], K. MORIYAMA[1], M. SANTOS[1], N. BOSAIPO[2], M. IYOMASA[1], M. ROSA[3,4];
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Abstract: Glutamate (Glu) has been shown to be the primary site of the abnormalities that occur in schizophrenia. Evidences have shown that Glu neurotransmission is decreased in the PFC and hippocampal formation in schizophrenics. Alterations on the mechanisms regulating this neurotransmission such as Glu receptors or Glu transporters may contribute to the development and/or maintenance of the schizophrenia. Rats reared in isolation from weaning have been used as experimental model for studying this disease and a decrease in Glu neurotransmission has already been demonstrated in this model. This study aimed at evaluating the changes on the expression of AMPA receptors (GluR1 and GluR2) and Glu transporters (EAAC1 and GLT1) in the PFC (primary somatosensory cortex) and entorhinal cortex (EC) of rats reared in isolation from weaning. Two groups of Wistar rats (n=5-8/each) were used. In both groups the pups remained with their mothers (6 pups/mother) until weaning (21 days - 40g) when they were allocated randomly to one of two conditions: grouped (housed 3/cage, handled 3 times/week) or isolated (housed individually, handled once/week for cleaning purpose) for 10 weeks. The animals were anaesthetized, perfused and their brains sectioned (40μm) in the PFC and EC for immunohistochemistry. The number of immunopositive cells (IC) or the optical density (OD) was quantified bilaterally in 3 sections/rat. Data were compared by Student t-test ($p<0.05$). Although a high density of GluR1- and GluR2-IC was found in the PFC, no change was induced by isolation rearing on the expression of both subunits in this area. However, isolation rearing induced a significant increase on the expression of EAAC1 in the PFC (38%, $p=0.017$), while no difference was found in the EC. The number of GLT1-IC did not change in the PFC of rats reared in isolation. The results for AMPA suggest that GluR1 and 2 are not involved on the reduction of the Glu neurotransmission in the PFC reported in schizophrenia. However, the involvement of AMPA in other sub-region of the PFC or the involvement of different Glu receptors in this mechanism can not be excluded. The results for EAAC1 suggest that this Glu transporter may contribute to the hypofunction of this neurotransmission in the PFC. Glutamate transporters may be a therapeutic target for the treatment of the schizophrenia in the future.
Support: Capes; FPA.

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Poster

707. Developmental Animal Models of Schizophrenia

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 707.22/W11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Maternal behavior of neonatal ventral hippocampus lesioned female rats

Authors: *C. P. SANCHEZ[1], S. GUZMAN-VELAZQUEZ[1], S. R. ZAMUDIO[1], G. FLORES[2], I. CAMACHO[2], G. M. MÁRQUEZ-PORTILLO[3], M. FLORES-JIMÉNEZ[3], Á. I. MELO[3]; [1]Physiol., Inst. Politécnico Nacional, Mexico, Mexico; [2]Lab. Neuropsiquiatría, Inst. de Fisiología-BUAP, Puebla, Mexico; [3]Ctr. de Investigación en Reproducción Animal, CINVESTAV-Laboratorio Tlaxcala, Univ. Autónoma de Tlaxcala., Tlaxcala, Mexico

Abstract: Neonatal ventral hippocampus lesion (nVHL) in rats has been used as a model to test the hypothesis that early neurodevelopment abnormalities lead to behavioral changes putatively linked to schizophrenia. Molecular and neurochemical alterations in prefrontal cortex, hippocampus and nucleus accumbens of nVH-lesioned animals suggest developmental reorganization of these structures that mimics this mental illness. As adults, male rats with nVHL show motor, social and cognitive abnormal dopamine-related behaviors. In contrast it is scarcely known how the nVHL affects the social behavior of female rats. In humans, it is known that schizophrenic mothers tend to be less behaviorally sensitive and responsive to their infants, and are scarce in social and physical contact with them. Also maternal infanticide is frequent. Therefore, to determine if nVHL female rats have disturbed mother-offspring social interactions, we characterized the maternal behavior in nVH-lesioned female rats. At postnatal day 7, female pups were anesthetized and their ventral hippocampus was damage with ibotenic acid (nVHL group) or vehicle (Sham group). As adults, females were mated with experimented males. At parturition day (PD1) the litters were culled to 8 pups and maternal behavior was recorded for 52 minutes at postpartum days 2, 6 and 12. Our results show that nVHL females spent less time retrieving the pups to the nest, and showed a longer latency to licking and less time licking the pups, than the Sham group. Also, nVHL females displayed low latency to nest building, and the frequency and duration was higher than Sham females. Furthermore, most of the nVHL females showed atypical retrieval (100%), atypical nest building (75%), and cannibalism (67%). Also, compared to Sham lesioned females, nHVL females displayed short latency and more frequency and duration of atypical retrieval and atypical nest building. These results suggest that maternal behavior of nVHL females is disrupted, but also present atypical behaviors that could be represent inappropriate social interactions.

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Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: JSPS Research Fellowships for Young Scientists

Title: Prenatal dexamethasone exposure induces asymmetrical ventriculomegaly and abnormal sensorimotor gating in mice

Authors: ***S. L. BENNER**[1], T. ENDO[1], K. ISHII[2], K.-I. KUBO[2], K. NAKAJIMA[2], C. TOHYAMA[1], M. KAKEYAMA[1,3];
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Abstract: Early life stress has epidemiologically been associated with the pathogenesis of psychiatric disorders including schizophrenia and bipolar disorder. This study demonstrates that prenatal stress in mice induces behavioral, histological and biochemical abnormalities which are seen in the schizophrenia and/or bipolar disorder patients. From gestational day (GD) 12.5 until birth, C57B/6J dams were administered daily with 20 μg dexamethasone (DEX) (0.5-1 mg/kg per day) mixed in pellet diet. The dosage caused an intrauterine growth restriction from which the mice recovered by adulthood (post 8 weeks), when they were subjected to biochemical, anatomical, and behavioral assessments. In mice prenatally exposed to DEX, hypothalamo-pituitary-adrenal axis feedback regulation was defective, and novelty response was suppressed, while no major cognitive retardation was detected. Importantly, neuroanatomical evaluation revealed that prenatal DEX exposure altered left-to-right ratio of the lateral ventriclar size. In particular, left ventricular enlargement was observed in DEX-exposed mice, a neuropathological phenotype found in schizophrenia and bipolar patients. We therefore examined whether DEX exposed mice possess sensorimotor gating abnormalities, a clinical symptom commonly observed in these psychiatric disorders, characterized by a decrease in inhibiting response to unimportant stimuli. Indeed, prepulse inhibition (PPI) was reduced in DEX exposed mice. These

results suggest that exposure to excessive stress hormone during late pregnancy may be in part responsible for causing pathological conditions observed in psychiatric disorders such as schizophrenia and bipolar disorder.

Disclosures: **S.L. Benner:** None. **T. Endo:** None. **K. Ishii:** None. **K. Kubo:** None. **K. Nakajima:** None. **C. Tohyama:** None. **M. Kakeyama:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Program#/Poster#: 707.24/W13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH

Title: A critical role of GABA release from ErbB4-positive interneurons in neural development

Authors: ***T. W. LIN**[1], D. YIN[2], A. BARIK[3], W.-C. XIONG[3], L. MEI[2];
[1]Neurosci., Georgia Hlth. Sci. Univ., Augusta, GA; [2]Dept. of Neurosci. and Regenerative Med., [3]Georgia Regents Univ., Augusta, GA

Abstract: ErbB4 is a receptor tyrosine kinase that can be activated by the trophic factor neuregulin 1 (NRG1). Both ErbB4 and NRG1 are susceptibility genes of schizophrenia. ErbB4 activation has been shown to promote GABA release from palvalbumin-positive interneurons and subsequent inhibition of pyramidal neuron activity. It has also been implicated in regulating pyramidal neurons in a cell autonomous manner. In this study, we investigated the role of GABA release from ErbB4-positive neurons by ablating vGAT (vesicular GABA transporter), a protein that loads GABA and glycine from the cytoplasm into synaptic vesicles. vGAT mutation wipes out spontaneous inhibitory postsynaptic currents. We crossed ErbB4::CreERT2 mice, where CreERT2 was expressed under the control of endogenous ErbB4 promoter, with vGATf/f mice where the second exon of vGAT was floxed. Resulting ErbB4::Cre-ER;vGATf/f (ErbB4-vGAT) mice were injected with tamoxifen, which activated the Cre recombinase and thus disrupted vGAT expression in ErbB4-positive neurons. We found that tamoxifen induction at E18 for 5 days impaired growth; pups were weak and smaller in size and died at age of 3 weeks. When the induction began at P9, mice developed spontaneous epilepsy and survived to 30-40 days of age. However, no epilepsy was observed in ErbB4-vGAT mice if they were injected with tamoxifen at P40. These results demonstrated a critical role of GABA release from ErbB4-positive

interneurons during neural development. Preliminary results suggested a role of the GABA release in the formation of soma-dendritic inhibitory synapses. Experiments are under way to investigating underlying molecular mechanisms.

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Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIDA Award DA14020

Sigma Xi Grants-in-Aid of Research

Title: Attenuation of compulsive cocaine-seeking in the neonatal ventral hippocampal lesion model of schizophrenia

Authors: *U. GYAWALI, S. HIRSH, A. H. BRADY;
Psychology, St. Mary's Col. of Maryland, St Marys City, MD

Abstract: Substance abuse is more prevalent in schizophrenic patients than in healthy individuals. A substantial overlap in the neuropathologies of schizophrenia and drug abuse suggests that vulnerability to addiction may be a core symptom of schizophrenia. The neonatal ventral hippocampal lesion (NVHL) animal model of schizophrenia produces multiple behavioral disturbances in adult rats that are consistent with psychopathology in schizophrenia including escalated self-administration of cocaine and methamphetamine. We recently observed that a subpopulation of NVHL rats also display compulsive drug seeking for cocaine, namely the continuation of drug-seeking despite negative consequences (footshock). Only a minority of normal rats show such compulsive behavior, and only after extended access to cocaine; the prefrontal cortex (PFC) is dysfunctional in these “addicted” rats. The PFC in NVHL animals is also dysfunctional, in particular, disinhibited and noisy, putatively resulting in poor decision-making and the observed compulsive seeking behavior. This study attempted to use the NMDA positive allosteric modulator CIQ to improve the PFC function in NVHLs and thus reduce their compulsive cocaine seeking. CIQ selectively targets NR2C/D subunits, expressed in prefrontal GABA interneurons but not pyramidal neurons. Animals received bilateral hippocampal

infusions of ibotenic acid (NVHLs) or artificial CSF (shams) on postnatal day (PD) 7. At PD60 or later, adult animals were trained to self-administer intravenous cocaine (0.75 mg/kg/infusion). Following acquisition, rats were tested for 10 days under punishment conditions where 5 of 11 daily drug trials terminated in foot-shock instead of cocaine delivery. Across days of punishment, NVHLs were observed to seek more cocaine and complete more trials than shams. When CIQ (5 mg/kg) was delivered intravenously on the 7th day of punishment, all animals decreased cocaine seeking compared to 6th day. No such differences were seen when vehicle was infused on the 4th day of punishment. On punishment days 8,9, and 10 when no treatment was administered, 80% of NVHLs resisted punishment, which suggests that they transitioned to compulsive drug seeking, while only 17% of shams were punishment-resistant. However, NVHLs were not differentially sensitive to unconditioned footshock, suggesting that the resistance to punishment was not due to changes in shock sensitivity. These data further support the result that NVHLs become compulsive drug seekers, and suggest that CIQ could be a therapeutic measure for addictive behavior in the NVHL model, and by extension, in schizophrenia.

Disclosures: **U. Gyawali:** None. **A.H. Brady:** None. **S. Hirsh:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Title: Dissecting the neuronal function of two different forms of DISC1 in *Drosophila*

Authors: ***B. LU**[1], L. SHAO[2], Y. ZHAO[1], L. WANG[1], Y. ZHONG[1];
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Abstract: Disrupted-In-Schizophrenia-1(DISC1), as one of the most intensively studied susceptibility genes in schizophrenia, was discovered in a Scottish pedigree in which a chromosomal translocation that breaks gene DISC1 segregates with psychiatric disorders. So it is considered that the variants of DISC1, particularly the truncated form, are highly associated with the inception and progress of schizophrenia. However, whether the schizophrenia pathological related effect of truncated DISC1 is due to the dominant negative manner of the wild-type DISC1 or the gain-of-function of the mutated DISC1 remained elusive, the dissection of which is hindered by the interferences of endogenous DISC1 in the prevailing mice models. Here, we tried to elucidate this problem by ectopically expressing different forms of DISC1 in *Drosophila melanogaster*, which is devoid of the endogenous counterpart of DISC1. We found that the truncated form of DISC1 was specifically involved in the functions of learning, neurotransmission and neurodevelopment.

Disclosures: B. Lu: None. **L. Shao:** None. **Y. Zhao:** None. **L. Wang:** None. **Y. Zhong:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Topic: A.10. Adolescent Development

Support: CONACYT # 156413 to Angel I. Melo

Title: Effect of early social isolation and artificial rearing in the copulatory behavior of male rats: Role of body and perineal tactile stimulation

Authors: C. AGUILAR[1], M. FLORES-JIMÉNEZ[2], V. RODRÍGUEZ-PIEDRACRUZ[1], R. LUCIO[3], *A. I. MELO[2];
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Abstract: In male rats, early social isolation through artificial rearing (AR) alters *ex copula* penile reflex and, *in copula* tests (four copulatory tests), increases mount and intromission latencies. Furthermore, only a few percentage (33%) of these males displayed the behavioral motor pattern of ejaculation. To assess whether AR rats need more opportunities to improve their

sexual performance, we increased the number of copulatory tests to eight. Additionally, to prevent the negative effects of AR, in others AR rats, we provided Tactile-perineal and/or body-stimulation during social isolation. Thus, male pups at 4 postnatal days were: 1) raised by their mother (MR-Control group), 2) separated from the nest and reared into an artificial rearing system (AR group), 3) AR provided with perineal-tactile stimulation with a wet painbrush, 5 times a day (AR-Perineal group), and 4) AR provided with perineal+body-tactile stimulation, 5 times a day (AR-Perineal-Body group). As adults (90-100 days of age) males were exposed to eight copulatory tests, with hormonal primed females. We found that the percentage of AR and AR-Perineal males that showed the ejaculatory pattern was significantly lower than that of the MR and AR-Perineal+Body males (0% and 25% vs 91% and 100%, respectively; $p<0.05$ for all comparisons). In addition, AR males displayed longer mount and intromission latencies in tests 3, 4 and 6, respect to the MR and AR-Perineal+Body males ($p<0.05$). Also, the number of intromissions of AR and AR-Perineal males, in some tests, was significantly lower, compared to MR and AR-Perineal-Body males ($p<0.05$). Results suggest that, despite a higher number of copulatory opportunities, the AR males did not improve their sexual performance. Furthermore, the replacement of tactile perineal plus body, but not perineal, stimulation prevents most of the effects of early social isolation. Partially supported by CONACYT project # 156413 to Angel I. Melo.

Disclosures: C. Aguilar: None. **V. Rodríguez-Piedracruz:** None. **A.I. Melo:** None. **R. Lucio:** None. **M. Flores-Jiménez:** None.

Poster

707. Developmental Animal Models of Schizophrenia

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Program#/Poster#: 707.28/W17

Topic:

Title: Impairments in working memory and conditioned reinforcement in the rat MAM model of schizophrenia

Authors: *D. YOUNG, T. A. LANZ, L. O'MALLEY, R. KOZAK, W. M. HOWE;
Pfizer Inc, Cambridge, MA

Abstract: The gestational methylazoxymethanol (MAM) rodent model has been shown to reproduce structural, neurochemical, neurophysiological, and cognitive aberrations hypothesized to contribute to schizophrenia (Lodge, 2013), yet the full extent to which this model recapitulates

the neuro-endophentype of the disorder remains to be determined. In the present studies, we investigated the performance of MAM rats on behavioral tasks that assess cognitive functions that are commonly impaired in the schizophrenic patient population, such as working memory. In the first study, we employed the continuous trial-unique delayed nonmatching-to-location (CTUNL) task. Rats were required to press a lighted square within a 3X5 grid on a touch screen monitor. Upon pressing, the animal is presented with reward. In all subsequent trials, the previously rewarded square and a new square in a random location were presented with the basic rule that the animal must press the new square to receive reward. MAM rats required significantly more training sessions to acquire task proficiency in the CTUNL task, although, if given enough time for performance to stabilize, working memory function was unaffected. To further investigate this apparent learning deficit in MAM rats, we next assessed their ability to learn basic stimulus-outcome association in a Pavlovian conditioned approach task, wherein the presentation of a novel conditioned stimulus (lever extension) was repeatedly paired with reward delivery. Over multiple days of training, MAM animals consistently displayed a significantly lower probability to engage in an approach behavior during lever extension, suggestive of a diminished capacity at even this basic form of learning. While previous studies from our lab and others have reported impairments in the working memory and attentional capacity of MAM animals in accordance with those observed in patients of schizophrenia, the results shown here indicate a fundamental learning deficit may also be a pervasive feature of this model. Ongoing studies assess the circuit bases of the behavioral disruptions observed here, with a particular focus on the role of aberrant local control of prefrontal networks by parvalbumin+ interneurons.

Disclosures: **D. Young:** A. Employment/Salary (full or part-time):; Pfizer Inc. Other; This work is part of the Innovative Medicines Initiative (IMI) project, Novel Methods leading to New Medications in Depression and Schizophrenia (NEWMEDS). **T.A. Lanz:** A. Employment/Salary (full or part-time):; Pfizer Inc. **L. O'Malley:** None. **R. Kozak:** A. Employment/Salary (full or part-time):; Pfizer Inc. **W.M. Howe:** A. Employment/Salary (full or part-time):; Pfizer Inc.

Poster

707. Developmental Animal Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH57440

Title: Amygdala hyperactivity in MAM model of schizophrenia and its reversal by peripubertal diazepam administration

Authors: *Y. DU[1], A. A. GRACE[2];
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Abstract: Stress is a major risk factor for schizophrenia that, when occurring in susceptible individuals, is proposed to lead to the onset of psychosis in late adolescence or early adulthood. Studies have shown that adolescents at high risk for schizophrenia demonstrated higher premorbid levels of anxiety and stress intolerance, which are associated with a range of prodromal symptoms and may predict the development of psychosis. Rats exposed during gestational day 17 to the mitotoxin methyl azoxymethanol acetate (MAM) exhibit as adults behavioral, pharmacological, and anatomical characteristics consistent with an animal model of schizophrenia. In addition, consistent with stress hypersensitivity in human at-risk subjects, peripuertal MAM rats showed abnormally high responses to stress and a heightened level of anxiety. As a pivotal region in stress responses, alterations in the amygdala may contribute to the stress hypersensitivity in MAM rats. Indeed, in current studies using *in vivo* extracellular recordings, we found that peripubertal (PD35-42) MAM rats (0.46± 0.04 Hz) showed a significantly higher spontaneous firing rate of basolateral amydala (BLA) neurons compared to Sal-treated rats (0.14± 0.02 Hz, $p<0.001$, t test). Given the evidence that stress responses early in life may be a factor in the transition to schizophrenia, we propose that alleviating stress in peripubertal MAM rats can prevent the emergence of schizophrenia-like symptoms in adulthood. We have demonstrated that peripubertal diazepam administration (PD31-40, daily, 5mg/kg) reversed dopamine neuron hyperactivity and attenuated heightened amphetamine-induced locomotion in MAM rats. In current studies, *in vivo* extracellular recording in BLA of adult MAM/ Sal rats with peripubertal diazepam(DZ)/ vehicle (Veh) treatment revealed significant effects of MAM ($p<0.001$) and diazepam ($p<0.01$, two-way ANOVA), in that BLA neurons in MAM:Veh rats (0.41± 0.04 Hz) showed a significantly higher spontaneous firing rate compared to Sal (Sal:Veh, 0.21± 0.03 Hz; Sal:DZ, 0.18± 0.04 Hz) and MAM:DZ (0.28± 0.04 Hz) ones. Moreover, in the elevated plus maze test for anxiety-like behavior, adult MAM rats showed a significantly higher level of anxiety than MAM:DZ and Sal rats: they spent significantly lower percentage of time in open arms (MAM:Veh. 7± 3; MAM:DZ, 34±4; Sal:Veh, 39± 10; Sal:DZ, 37± 5; $p<0.01$) and made lower percentage of open arm entries (MAM:Veh, 14± 6; MAM:DZ, 38± 5; Sal:Veh, 40± 9; Sal:DZ, 38± 3; $p<0.05$, two-way ANOVA). Therefore, peripubertal treatment with diazepam reversed both the increased BLA neuron firing and increased anxiety levels observed in MAM-treated rats.

Disclosures: Y. Du: None. **A.A. Grace:** None.

Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 708.01/W19

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: ERANET-NEURON JTC2012

Title: Oligodendrocytes and cerebral white matter integrity in depressed suicides

Authors: *A. TANTI, M. WAKID, M. A. DAVOLI, G. TURECKI, N. MECHAWAR;
McGill Group For Suicide Studies, Montreal, QC, Canada

Abstract: Major depressive disorder (MDD) is increasingly associated with alterations in the organization of cerebral white matter tracts, as evidenced by neuroimaging studies (DTI). It remains to be determined, however, whether these changes reflect myelin abnormalities and impairments in oligodendrocyte function. The current postmortem study aims at characterizing the cellular and molecular features of prefrontal cortical white matter in depressed suicides and matched controls. Brain samples from well-characterized depressed suicides (n=15) and matched sudden-death controls with no psychiatric history (n=15) were obtained from the Douglas-Bell Canada Brain Bank and processed for immunohistochemistry. Stereological counts of immature and myelinating oligodendrocytes immunolabeled for Olig2 and Nogo-A, respectively, were performed. Myelin main protein constituents (MBP, CNPase, MAG and MOG) and genes involved in myelin formation and oligodendrocyte function were also assessed by immunoblotting and qPCR to gain insight on myelin integrity at the cellular and molecular level. Preliminary results indicate subtle changes in myelin composition and in the distribution of oligodendrocytes in the prefrontal cortex white matter of depressed suicides, which together may underlie the connectivity changes observed in some imaging studies. Myelination of axonal fibers is a critical step in the functional maturation of the brain. As such, alterations of white matter and myelin integrity are likely to induce impairments in cognitive and affective functioning that could precipitate the onset of psychiatric disorders or participate in their complex pathophysiology. Further work is required to understand the mechanisms leading to impaired oligodendrocytes function in MDD.

Disclosures: **A. Tanti:** None. **M. Wakid:** None. **M.A. Davoli:** None. **G. Turecki:** None. **N. Mechawar:** None.

Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 708.02/W20

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: CIHR

AFSP

Title: GDNF signaling in the amygdala of depressed suicides

Authors: *M. E. MAHEU, J. P. LOPEZ, M. A. DAVOLI, L. CRAPPER, G. TURECKI, C. ERNST, N. MECHAWAR;
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Abstract: Background: Glial cell line-derived neurotrophic factor (GDNF) is a potent pro-survival factor for neurons, and mediator of structural plasticity. Despite substantial research having implicated GDNF in the aetiology of depression, and peripheral GDNF expression has been shown to vary with mood state, little is known about the state of GDNG signaling in the role in the brain of individuals suffering from major depression. Methods: Expression of GDNF signaling molecules (GDNF, GFRα1, Ret, and NCAM) was assessed at the protein (immunoblotting) and mRNA level (qPCR) in the basolateral amygdala (BLA) of depressed suicides (DS) and matched sudden-death controls (CTRL). Candidate regulatory microRNAs (miRNAs) were identified in silico and measured by qPCR. The effect of candidate miRNA over-expression on protein, gene expression, and GDNF signaling was then assessed *in vitro*. Results: Although GDNF was found to be unaltered in the BLA, DS subjects displayed decreased expression of its receptor GFRα1. Reductions in GFRα1 protein were associated with decreased expression of one particular transcript (GFRα1a), and increased expression of miRNAs predicted to bind to GFRα1a with high affinity (miR-511 and miR-340). Transfection of miR-511 into human neural progenitor cells (NPCs) resulted in the specific repression of GFRα1a and altered GDNF signaling, including changes in immediate early gene, Akt, and MAPK activity. Conclusion: Depressed suicides display reduced expression of the GDNF receptor GFRα1 in the BLA. This reduction appears to be isoform-specific, mediated by microRNAs, and sufficient to alter GDNF signaling. Taken together, our results suggest that GDNF signaling is likely impaired in the BLA of depressed subjects.

Disclosures: M.E. Maheu: None. **J.P. Lopez:** None. **M.A. Davoli:** None. **L. Crapper:** None. **G. Turecki:** None. **C. Ernst:** None. **N. Mechawar:** None.

Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

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Program#/Poster#: 708.03/W21

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Pritzker Neuropsychiatric Disorder Consortium

Title: Differential gene expression patterns in various sub-nuclei of human amygdala

Authors: *V. SHARMA[1], M. HAGENAUER[2], S. CHAUDHURY[1], R. C. THOMPSON[1], R. M. MYERS[3], A. F. SCHATZBERG[4], J. D. BARCHAS[5], W. E. BUNNEY[6], H. AKIL[1], S. J. WATSON[1];
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Abstract: The amygdala plays an important role at the interface of cognition and emotion, in part due to its connectivity to anterior midline structures and the temporal subcortical regions. This elaborate repertoire of functions pivots around its various sub-nuclei and their extensive connections. However, there is no consistent anatomical description of these sub-nuclei across various species, especially in humans. To date, there have been no studies undertaken to show the gene expression pattern in these individual sub-nuclei. This may not only be relevant in terms of understanding the functions of human amygdala but also the role it plays in pathophysiology of various psychiatric disorders. In the present study we report the gene expression in the individual sub-nuclei of the amygdala in normal healthy control subjects. Human brain samples of 16 healthy control subjects were obtained from the Brain Donor Program at the University of California, Irvine. The blocks containing the amygdala region were cryo-sectioned at 10µm thickness. The location and orientation of various sub-nuclei in these sections were identified using acetylcholinestrase staining. Using laser capture microscopy, total RNA was extracted from various sub-nuclei and processed for microarrays (Illumina Bead Chip Array). The microarray data from each sub-nucleus was rigorously analyzed using statistical procedures to reduce the effects of important confounding factors and sources of noise, including pre- and post-mortem variables, sex, age, and RNA integrity. Cross-regional comparison will be done to explore the signature gene expression pattern for individual sub-nuclei. These differential expression patterns of various genes will help to make a comprehensive anatomical map defining

the molecular functioning of individual sub-nuclei of the human amygdala and to identify the role played by each sub-nucleus in extensive functions of amygdala.

Disclosures: **V. Sharma:** None. **M. Hagenauer:** None. **S. Chaudhury:** None. **R.C. Thompson:** None. **R.M. Myers:** None. **A.F. Schatzberg:** None. **J.D. Barchas:** None. **W.E. Bunney:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 708.04/W22

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: UICentre

Title: GluA2 RNA editing is increased in major depression and suicide

Authors: ***E. NWABUISI-HEATH**[1], A. GUIDOTTI[2], E. DONG[2], K. RATIA[3], M. SODHI[4]; [1]Dept. of Pharm. Practice, Univ. of Illinois at Chicago, Chicago, IL; [2]Dept. of Psychiatry, [3]Res. resources core, High-throughput screening, [4]Departments of Pharm. Practice and Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Major depressive disorder (MDD) is debilitating, and afflicts up to 20% of the population worldwide. A large proportion of patients with MDD attempt or commit suicide. The molecular mechanisms that underlie MDD remain unknown, hindering the development of effective antidepressant therapies. Several independent laboratories have reported increased RNA editing of the 5-HT2C receptor in the postmortem brains of MDD patients who commit suicide. Consistent with this previous work, we have observed increases in the RNA editing of both GluA2 and 5-HT2C in the dorsolateral prefrontal cortex of MDD subjects who committed suicide. However, it is not clear if altered RNA editing plays a role in the pathophysiology of MDD and suicide. Multiple studies in humans and animals show that early life stress is a predictor of MDD. Therefore, to complement our work in postmortem MDD subjects, we are investigating RNA editing in a mouse model of depression created by prenatal stress (PRS). To determine whether a generalized or specific alteration in RNA editing may underlie depressed behavior in PRS mice, the expression of RNA editing enzymes (ADAR1, 2 and 3) are being analyzed. In parallel, we have employed a high-throughput screening assay for GluA2 RNA editing in yeast to identify inhibitors of RNA editing from the Prestwick Library of FDA

approved drugs. Counter-screened drugs that reduced GluA2 RNA editing include currently prescribed antidepressants such as reboxetine and fluoxetine. Identifying a specific molecular probe for RNA editing will aid our understanding of RNA editing effects on depressive behavior in PRS mice, and will facilitate the development of new therapeutic treatment strategies for MDD and suicide.

Disclosures: **E. Nwabuisi-Heath:** None. **A. Guidotti:** None. **E. Dong:** None. **K. Ratia:** None. **M. Sodhi:** None.

Poster

708. Mood Disorders: Human Postmortem

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH077159

Title: Selective dysregulation of dendritically-targeted BDNF transcripts and interneuron markers in the dorsolateral prefrontal cortex of major depressive disorder

Authors: *H. OH, D. A. LEWIS, E. L. SIBILLE;
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Abstract: Introduction. A parallel downregulation of brain-derived neurotrophic factor (BDNF) and somatostatin (SST), a marker of GABA interneurons which target the distal dendritic compartment of pyramidal cells, has been observed in multiple brain areas of depressed subjects. BDNF is an upstream regulator of SST expression, however, the underlying mechanism remains unclear. Recent animal studies report that BDNF transcript variants have distinct cellular localization and that selective knockdown of dendritically-targeted BDNF splice variants is sufficient to induce anatomical and behavioral abnormalities. The aim of this study was to investigate the specificity and correlation of expression changes for specific BDNF transcripts to interneuron markers in major depressive disorder (MDD), using postmortem human brain samples. Method. We identified the top 200 genes positively correlated with BDNF using microarray data obtained from orbitofrontal cortex (BA47) of control subjects (n=209, 16-96 year old, no signs of psychiatric or neurological diseases). With qPCR, we determined the expression level of BDNF transcript variants and synaptic markers in the dorsolateral prefrontal cortex (BA46) of 19 pairs of male and female subjects were analyzed, consisting of MDD and

control matched for sex, race, and as closely as possible for age, postmortem interval and brain pH. Results. (1) BDNF is strongly linked to genes related to the synaptic compartment and function, especially GABRA5. (2) Reduced expression of dendritic-targeting interneuron markers parallels low dendritic BDNF transcripts in MDD. (3) Long 3' UTR of BDNF is highly correlated to the depression-regulated genes, but not to the genes unaltered in disease state. Discussion. Our results provide evidence that MDD-related downregulation of dendritic BDNF contributes to selective impairment of dendritic-targeting interneurons in depressed subjects.

Disclosures: H. Oh: None. **D.A. Lewis:** None. **E.L. Sibille:** None.

Poster

708. Mood Disorders: Human Postmortem

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Support: VA Merit Grant BX001829

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Title: A unique gene expression signature associated with serotonin 2C receptor RNA editing in the prefrontal cortex and altered in suicide

Authors: *S. DRACHEVA[1,3], A. F. DI NARZO[4], A. KOZLENKOV[1,5], P. ROUSSOS[2,6], K. HAO[4], Y. HURD[3], D. A. LEWIS[7], E. SIBILLE[7], L. J. SIEVER[1,3], E. V. KOONIN[8];
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Abstract: Editing of the pre-mRNA for the serotonin receptor 2C (5-HT2CR) by site-specific adenosine deamination (A-to-I pre-mRNA editing) substantially increases the functional plasticity of this key neurotransmitter receptor and is thought to contribute to homeostatic mechanisms in neurons. 5-HT2CR mRNA editing generates up to 24 different receptor isoforms. The extent of editing correlates with 5-HT2CR functional activity: more highly edited isoforms exhibit the least function. Altered 5-HT2CR editing has been reported in post-mortem brains of suicide victims. Here we report a comparative analysis of the connections among 5-HT2CR editing, genome-wide gene expression and DNA methylation in FACS-isolated neuronal nuclei (measured by massive parallel sequencing, RNA-Seq, and Infinium Human Methylation450K array, respectively) in suicide victims, individuals with major depressive disorder (MDD) and non-psychiatric controls. We investigated autopsy prefrontal cortex brain specimens from two independent cohorts; Cohort1: 22 suicides and 29 non-suicides without antemortem psychiatric diagnosis (DX); Cohort2: MDD patients with (N=10) or without (N=24) suicide, and non-suicides without psychiatric DX (N=31). The results confirm previous findings of an overrepresentation of highly edited mRNA variants (which encode hypoactive 5-HT2CR receptors) in the brains of suicide victims. A large set of genes for which the expression level is associated with editing was detected. This signature set of editing-associated genes is significantly enriched for genes that are involved in synaptic transmission, genes that are preferentially expressed in neurons, and genes whose expression is correlated with the level of DNA methylation. Notably, we report that the link between 5-HT2CR editing and gene expression is disrupted in suicide victims. The results of this work imply that the postulated homeostatic function of 5-HT2CR editing is dysregulated in the brains of suicide victims. The increased editing (or more precisely, relative paucity of unedited 5-HT2CR variants) could be a synergistic effect of suicide-associated mutations and environmental stimuli.

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Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

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Program#/Poster#: 708.07/W25

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: CIHR grant MOP-231096

Title: Investigating inflammatory markers in the choroid plexus of depressed suicides

Authors: *J. DEVORAK[1,2], S. G. TORRES-PLATAS[1,2], M. DAVOLI[1], N. MECHAWAR[1,2]; [1]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Several lines of evidence, from animal models to clinical studies, indicate that increased levels of circulating pro-inflammatory cytokines can trigger or accompany depressed mood. While this likely involves cerebral inflammation, there exists little supporting evidence in the literature. The choroid plexus (ChP), a highly vascularized tissue that produces cerebrospinal fluid and lacks a blood-brain-barrier, is at the interface of peripheral and central immune responses. This postmortem study aims to investigate and compare cell populations as well as molecules know to be implicated in central and peripheral inflammatory responses between depressed suicides and psychiatrically healthy controls. Preliminary analyses of ChP macrophages immunostained for ionized calcium-binding adaptor molecule 1 (Iba-1) in samples from both subject groups indicate that, in general, the morphology and distribution of ChP Iba1-immunoreactive (IR) cells are distinct from those previously described in cerebral cortex (Torres-Platas et al., 2014, J Neuroinflammation, 11:12). Iba1-IR cells in the ChP overwhelmingly display an amoeboid morphology. Furthermore, the morphological features of Iba1-IR cells in the ChP vary as a function of their location within the tissue. While amoeboid-like cells are observed throughout the ChP, including the stroma, villi, and extensive vascular network, variations in this amoeboid-like morphology are observed between ChP compartments. Iba1-IR cells in the stroma often display one or two unramified processes, whereas cells localized within the villi or in close proximity to blood vessels are more frequently devoid of processes. Microglia-like Iba1-IR cells displaying multiple processes appear confined to the stroma. Further investigation of macrophage subtypes and their comparison between depressed suicides and controls will also be presented.

Disclosures: J. Devorak: None. **S.G. Torres-Platas:** None. **M. Davoli:** A. Employment/Salary (full or part-time):; Douglas Mental Health University Institute. **N. Mechawar:** 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.; CIHR.

Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

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Program#/Poster#: 708.08/W26

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Ministry of Science (SAF07-61862)

Ministry of Economy and Competitivity (SAF2011-25020)

Title: Role of Wnt/β-catenin and mTOR pathways in antidepressant response in human brain

Authors: *F. PILAR-CUELLAR[1,2,3], R. VIDAL[1,2,3], R. LINGE[1,2,3], B. ROMERO[1,2,3], A. DIAZ[1,2,3], E. CASTRO[1,2,3], E. M. VALDIZAN[1,2,3], A. PAZOS[1,2,3];
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Abstract: Classical monoamine hypothesis of depression implies a decrease in brain neurotransmitters as serotonin and noradrenaline, which are increased after chronic antidepressant treatment. New hypotheses suggest the impairment of neuroproliferative/neuroplastic pathways involved in these diseases, which are upregulated by the use of antidepressants. However, although animal models have been extensively studied, few data have been reported in the same set of human brain samples regarding the activation level of these diverse pathways. Here we have studied the protein and mRNA expression of the neurotrophic factor BDNF, the activation of Wnt/β-catenin pathway (GSK3β phosphorylation and β-catenin levels), and mTOR pathway activation (mTOR and 4EBP1 phosphorylation) in frontal cortex from human brain samples dissected at autopsy from 16 suicide victims and 16 control subjects. In Wnt/ β-catenin pathway, we found a significant decrease in β-catenin protein level in brain samples from depressed suicide free of antidepressant treatment (AD-free group) compared to control group ($p<0.01$), but not in the samples from depressed suicide with antidepressant treatment (AD-treated group). β-catenin mRNA levels were no changed in the different groups. In addition, the phosphorylation of GSK-3β (Ser9) (ratio p-GSK-3β/GSK-3β), was significantly increased in AD-treated group compared to controls ($p<0.05$) and AD-free groups ($p<0.05$). Regarding mTOR pathway activation, mTOR phosphorylation (ratio p-mTOR/mTOR) was increased in brain samples from AD-treated group compared to control ($p<0.05$) and AD-free groups ($p<0.05$). In parallel to this increased activation, a clear tendency to the increase of mTOR mRNA levels in AD-treated group compared to the other groups was observed. In addition, we found a decrease in the phosphorylation of 4E-BP1 protein (ratio p-4E-BP1/4E-BP1) in brain samples from depressed suicide. A reduction in mRNA levels was also observed for AD-treated ($p<0.05$) and AD-free groups ($p<0.05$) compared to control group. In addition, BDNF levels were also decreased in AD-free group ($p<0.05$ vs the control group) as previously reported, with a partial recovery of this neurotrophic factor level after antidepressant treatment. These results indicate the impairment of neuroproliferative/neuroplastic pathways as Wnt/β-catenin and BDNF in major depression. These pathways are partially restored after antidepressant treatment, together with the activation of the mTOR pathway.

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Poster

708. Mood Disorders: Human Postmortem

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Support: VIEP-BUAP Grant FLAG/IND14

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Title: Increased incidence of adenomas and differences in zinc presence in adenopituitary of young adult suicide victims

Authors: *H. TENDILLA, SR[1], P. AGUILAR-ALONSO[2], F. DE LA CRUZ[3], F. GARCÍA-DOLORES[4], E. BALTAZAR-GAYTÁN[2,1], G. FLORES[1];
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Abstract: Suicide is considered a public health problem and it is caused by several factors such as the presence of psychopathology, alcohol and other drugs dependence or early-life events that generate acute stress (abuse, humiliation, social rejection, etc.). From these, the presence of psychopathology, specifically major depressive disorder (MDD), has the strongest relationship to suicide risk or to develop suicide behavior. The hypothalamic-pituitary-adrenal (HPA) axis plays an important role in the depressive disorder since is the major stress regulatory system, it has been reported an hyperactivity of the HPA axis as well as increased size and weight of the pituitary gland in a significant percentage of suicidal behavior patients, also it has been reported the presence of pituitary adenomas in suicide victims. Recently it has been proposed the zinc as a marker of affective disorders, several reports indicated decreased serum zinc concentration in depressed patients, this could mean a dysregulation in the homeostasis of zinc, an important mineral in the secretion of hormones in the pituitary. This research aimed to determine the

incidence of adenomas and zinc in the pituitary of suicide victims compared with controls (patients who died from other causes). Slices of pituitary was HE stained for analyze the presence of adenomas, and zinquin and dithizone staining to reveal the zinc presence. Although it has been reported the presence of adenomas in non-suicide victims we found an increased incidence of pituitary adenomas in suicide victims compared with controls, localized in the adenopituitary with acidophilic characteristics. On the other hand we found differences both in free and compartmentalized zinc in the pituitary between suicide victims and control patients. Our results support the suggestion that the presence of pituitary adenomas is a risk of factor for suicide.

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Poster

708. Mood Disorders: Human Postmortem

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MH071537

Title: The functional serotonin 1a receptor promoter polymorphism, rs6295, is associated with psychiatric illness and differences in transcription

Authors: *Z. R. DONALDSON[1], B. LE FRANCOIS[2], T. L. SANTOS[3], M. BOLDRINI[4], V. ARANGO[4], C. A. STOCKMEIER[5], K. J. RESSLER[6], P. R. ALBERT[2], R. HEN[4];
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Psychiatric Inst., New York, NY; [4]Columbia University/New York State Psychiatric Inst., New York, NY; [5]Univ. of Mississippi Med. Ctr., Jackson, MS; [6]Emory Univ., Atlanta, GA

Abstract: The G/C single nucleotide polymorphism (SNP) in the serotonin 1a receptor promoter, rs6295, has previously been linked with depression, suicide, and antidepressant responsiveness, indicating that it may contribute to the risk for mental illness and differences in treatment outcomes. *In vitro* studies suggest that rs6295 may have functional effects on the expression of the serotonin 1a receptor gene (HTR1A) through altered binding of a number of transcription factors, including Deaf1/NUDR and the development-specific factors, Hes1 and Hes5. In order to further explore the relationship between rs6295, mental illness, and gene expression, we undertook dual epidemiological and biological studies. First, we genotyped a cohort of 1412 individuals from the Grady Trauma Project. Within this predominantly black, highly traumatized cohort, we found that the rs6295G allele is associated with increased risk for psychiatric hospitalization, and in females, with an increased risk for post-traumatic stress disorder. In conjunction, we investigated the potential impact of rs6295 on HTR1A expression in post-mortem human brain tissue. Using allelic imbalance assays, we found that the rs6295C allele is associated with increased HTR1A expression compared with the rs6295G allele in the prefrontal cortex but not in the midbrain or hippocampus of control subjects. Further, in the fetal cortex, rs6295C is associated with increased expression as early as gestational week 17 in humans. Finally, we found that relative increase in expression from the rs6295C allele was not found in the prefrontal cortex of subjects with Major Depressive Disorder who committed suicide, indicating that normal patterns of transcription may be disrupted in suicide. Our data support *in vitro* studies showing that in adulthood, rs6295 may have region-specific effects on HTR1A expression. Moreover, our results suggest that effects of the polymorphism may be occurring during both gestational development and adulthood. These findings further support a role for rs6295 in modulating HTR1A expression and contributing to psychiatric illness.

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Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 708.11/W29

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: CIHR MOP 84291

CIHR MOP 93775

NIH 8914

Title: A truncated form of tropomyosin-related kinase B (TrkB) is deregulated in frontal cortex of suicide completers through molecular mechanisms involving its 3'UTR DNA sequence

Authors: *G. MAUSSION[1], J. YANG[1], V. YERKO[1], M. SUDERMAN[2], A. DIALLO[1], C. NAGY[1], M. ARNOVITZ[1], P. BARKER[3], C. ERNST[1], N. MECHAWAR[1], G. TURECKI[1]; [1]Dept. of Psychiatry, Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]Departments of Pharmacol. and Therapeut., McGill Univ., Montreal, QC, Canada; [3]Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: TrkB-T1 is a BDNF receptor lacking a tyrosine kinase domain that is highly expressed in astrocytes and regulates BDNF-evoked calcium transients. Previous studies indicate that downregulation of TrkB-T1 in frontal cortex may be involved in neurobiological processes underlying suicide. Here we want to review the different mechanisms involved in the expression changes of neurotrophins in a context of suicide, and particularly those involving the TrkB-T1 3'UTR sequence based on studies that have recently been published. In fact using microarray approaches assessing the genome wide microRNA profile and the whole TrkB gene methylation pattern in the frontal cortex of suicide completers with low TrkB-T1 expression, significant differences were characterized. MicroRNAs Hsa-miR-185* and Hsa-miR-491-3p were upregulated in suicide completers with low expression of TrkB.T1 after correction for multiple testing. Bioinformatic analyses revealed five putative binding sites for the DiGeorge syndrome linked microRNA Hsa-miR-185*in the 3'UTR of TrkB-T1 but none for Hsa-miR-491-3P. The increase of Hsa-miR-185* in frontal cortex of suicide completers was validated and an inverse correlation between Hsa-miR-185* and TrkB-T1 expression was observed. The methylation array on the full TrkB gene has also revealed five probes located in the TrkB-T1 3'UTR region and found hypermethylated in the frontal cortex of suicide completers. These results were validated for four CpGs sites spanning a 150 bp sequence by cloning and Sanger sequencing bisulfite treated DNA. Furthermore an inverse correlation between TrkB-T1 expression and methylation level at those sites was found. *In vitro* functional analyses (silencing and overexpression of microRNA Hsa-miR-185* as well as *in vitro* methylation assays) confirm the role of microRNA binding and of methylation in the modulation of TrkB-T1 expression. These data suggest that TrkB-T1 3'UTR sequence may play a significant role in the important decrease of cortical TrkB-T1 expression observed among suicide completers. Those results, taken together with other investigations, reinforce the idea that molecular dysregulations of neurotrophin pathway are key factors in mood disorders.

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Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Pritzker Neuropsychiatric Research Consortium

Title: Comparative expression of neuropeptide processing enzymes in the postmortem hippocampus of control vs. depressed subjects

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Abstract: Inactive peptide precursors are converted into biologically active neuropeptides through a series of enzymatic steps, the first of which is carried out by the family of proprotein convertases (PCs). Most members of the PC family cleave inactive peptide precursors C-terminal to paired basic amino acid residues (e.g., Lys/Arg). Two members of the PC family, PC1 and PC2, are stored in secretory granules and act on peptide precursors that are processed in the regulatory secretory pathway. Early in the secretory pathway, the activity of PC1 and PC2 can be blocked when bound by their associated endogenous inhibitors, proSAAS (PC1) and 7B2 (PC2). We have previously described the distribution of these enzymes in the posterior hippocampus of control subjects with no reported history of psychiatric disorders (n=9). Here, we examined the distribution of these enzymes in the same brain regions of subjects with a history of major depressive disorder (MDD; n=17) and subsequently compared differences in mRNA expression between control and MDD subjects. Frozen 10µm sections through the posterior hippocampus of normal control and MDD subjects were processed for *in situ* hybridization histochemistry using radiolabeled cRNA probes for PC1, PC2, proSAAS and 7B2, and mRNA expression was subsequently measured in the dentate gyrus (DG) and CA subfields (CA1, CA2 and CA3) of this

region. Interestingly, we observed selective differences in the expression of these processing enzymes that appear only within certain hippocampal subfields. Studies are currently underway to examine the colocalization of these PCs/PC inhibitors with neuropeptides (e.g., cholecystokinin, neuropeptide Y, etc.,) in the posterior hippocampus as well as other associated brain regions. Overall, examining differences in neuropeptide processing in the brains of control vs. depressed subjects may provide a better understanding of how changes in these molecules are related to MDD.

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Poster

708. Mood Disorders: Human Postmortem

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Conte Center Grant #L99MH60398

The Pritzker Neuropsychiatric Research Consortium

Title: Analysis of hypothalamic substance p mrna in major depressive disorder

Authors: *D. M. KROLEWSKI[1], A. MEDINA[1], R. M. MYERS[2], A. SCHATZBERG[3], J. D. BARCHAS[4], W. E. BUNNEY[5], H. AKIL[1], S. J. WATSON[1];
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Abstract: Substance P (SP) is a well conserved neuropeptide encoded by the preprotachykinin-A gene. Through activation of G-protein coupled receptor signaling systems, SP has been shown to regulate a diverse array of physiological and behavioral processes including modulation of stress responses related to depression and anxiety. The highly interconnected hypothalamus, which plays a role in major depressive disorder (MDD) via an elevated hypothalamic pituitary adrenal-axis (HPA-axis), exhibits particularly enriched SP gene expression. However, the potential role of hypothalamic SP in MDD has not yet been investigated. Therefore, the aim of current study is to better understand the anatomical relationship with other locally expressed hypothalamic

signaling molecules in addition to evaluating potential quantitative SP expression differences between controls and MDDs. To accomplish this, frozen hypothalamic blocks from normal controls and MDDs were sectioned at 10µm on a cryostat and *in situ* hybridization (ISH) was subsequently performed utilizing a radiolabeled SP cRNA probe. Then, with the aid of a neuropeptide mRNA-based hypothalamic map generated by the Pritzker Neuropsychiatric Research Consortium (Krolewski et al., 2010), individual subjects were anatomically aligned to simultaneously view multiple cRNA probes. Our preliminary observations include visualized regional overlap of SP mRNA with glutamate decarboxylase isoform 67 (GAD67) mRNA in the anterior hypothalamic nucleus as well as that of melanin-concentrating hormone (MCH) and orexin (ORX) in both the lateral and posterior hypothalamic areas. Quantitative assessment within neuropeptide-specific anatomical locations is currently ongoing. Results obtained from the present analysis examining SP mRNA distribution and its possible altered regulation in MDD will further the understanding of neuropeptide function in psychiatric illness.
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Disclosures: D.M. Krolewski: None. **A. Medina:** None. **W.E. Bunney:** None. **R.M. Myers:** None. **J.D. Barchas:** None. **A. Schatzberg:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

708. Mood Disorders: Human Postmortem

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: FRSQ

CIHR

NSERC

Title: Temporally and subregionally specific involvement of hippocampal neuregulin-1/ErbB3 signaling in neurogenic and affective regulation

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Abstract: Background Hippocampal neurogenesis has been implicated in the mechanism of antidepressant action, and neurotrophic factors can mediate neurogenic changes underlying these effects. The neurotrophic factor neuregulin-1 (NRG1) is involved in many aspects of brain development. However, little is known about the influence of NRG1 on neurodevelopmental processes in the mature hippocampus. These experiments examine the potential role of NRG1-ErbB signaling in hippocampal neurogenesis, affective behaviour, and psychopathology. **Methods** Adult mice were given subcutaneous NRG1 or saline to assess dentate gyrus (DG) proliferation, survival, differentiation, morphology, and neurogenesis, and underwent behavioral testing. Expression of NRG1 receptors in newborn DG cells was assessed at various time points. ErbB-expressing progenitor cell phenotype was characterized. DG dissections were used for ELISA. We extended these findings into psychiatrically-relevant human subjects, examining hippocampal NRG1/ErbB gene expression and methylation in suicide completers and controls. **Results** Subchronic peripheral NRG1β administration selectively increased cell proliferation and neurogenesis (but not survival, differentiation, or immature neuronal morphology) in the ventral DG. These effects may have been mediated by ErbB3 receptors, expressed by newborn cells from division to maturity and colocalized with SOX2 in the subgranular zone. NRG1 increased ventral DG ErbB3 phosphorylation. Four weeks (but not acutely) after treatment, animals displayed antidepressant-like behavior. In humans, hippocampal ErbB3 expression was reduced in suicides, which was not due to ErbB3 methylation, although antidepressants induced methylation changes. **Conclusions** The neurogenic and affective effects of NRG1β are subregionally and temporally specific (affecting proliferation and neurogenesis in the ventral hippocampus and associated antidepressant-like behavior, but not neuronal differentiation, survival, or morphology). These effects potentially occur through NRG1-ErbB3 signaling, given ErbB3's expression profile and increased phosphorylation in the ventral DG following NRG1 administration, and human psychiatrically-relevant subjects show reduced hippocampal ErbB3 expression. Given the implication of NRG1 in psychopathology and the importance of ventral hippocampal neurogenesis in emotion-related behavior and response to stress and antidepressants, these data have potential relevance to the understanding of affective regulation and psychiatric disorders. *Supported by: FRSQ, CIHR, NSERC*

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Poster

708. Mood Disorders: Human Postmortem

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

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Post-doctoral fellowship from the Canadian Institutes of Health Research (PEL)

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Title: Epigenetic regulation of the anterior cingulate cortex by childhood maltreatment

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Abstract: Childhood maltreatment (CM) is global problem of significant proportion that affects children of all ages, race, and socio-economic backgrounds. There is a strong relationship between CM and mental health outcomes throughout the lifespan. Accordingly, CM is among the strongest predictors of psychiatric pathology, such as depression and suicide. Understanding how early-life experiences can affect behavior and mental health over the lifespan is a major challenge. Epigenetic mechanisms, in particular DNA methylation, have recently emerged as a form of genomic plasticity that has the potential to modify gene expression over extended periods of time. We hypothesize that CM contributes through epigenetic and gene expression changes to the risk of depression and suicide into adulthood. We focus our analysis on the anterior cingulate cortex (ACC), a brain region that crucially regulates emotional states and that is strongly responsive to adverse life experiences. Using brain post-mortem tissues available through the Douglas-Bell Canada Brain Bank, we compare 27 depressed suicide completers with a history of CM, with 26 psychiatrically normal individuals with no history of CM. Information on psychiatric diagnoses and history of CM were obtained for all subjects through psychological autopsies. We characterized genome-wide DNA methylation patterns in the ACC, using Reduced Representation Bisulfite Sequencing. Results uncover a collection of a hundred genomic regions, where significant differential DNA methylation is detected following multiple-testing correction. To assess the functional impact of DNA methylation changes associated with CM, we performed a genome-wide transcriptomic analysis using RNA-Sequencing in the same cohort of subjects. We are currently comparing gene expression changes associated with a history of CM with results from our epigenetic study. DNA methylation differences that show evidence of potential

impact at the transcriptional level will be further investigated. Overall, the present project will allow us to robustly and specifically identify novel candidate pathways epigenetically induced by CM in the brain, and to propose possible avenues for intervention.

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Poster

709. Mood Disorders: Animal Models I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 709.01/W34

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Abnormal gamma oscillations in a genetic rat model of depression

Authors: P. STIENEN[1,2], A. RACHALSKI[1,2], M. LINDSKOG[3], *E. ÅBERG[1,2];
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Abstract: Mood disorders have been clinically associated with abnormalities in gamma oscillations [1]. These oscillations are generated by combined networks of pyramidal neurons and gamma-aminobutyric acid (GABA) interneurons [2]. In the present study, we explored abnormalities in gamma oscillations in a genetic rat model of depression, the Flinders Sensitive Line (FSL) rat, compared to a standard laboratory rat, the Sprague Dawley (SD). To this aim, fronto-parietal electro-encephalography (EEG) was recorded in FSL (*n*=8) and SD (*n*=8) rats following the administration of vehicle, 10 and 30 mg/kg of the N-methyl d-aspartate receptor (NMDA-R) antagonist ketamine. Ketamine application is thought to lead to an inhibition of GABA interneurons. The subsequent disinhibition and increased firing of pyramidal neurons allows these neurons to become more entrained in gamma oscillatory activity [3]. Ketamine increased dose-dependently gamma power in both FSL and SD rats. However, the gamma power increase was much larger in the FSL rats suggesting that FSL rats under normal conditions, present abnormalities in gamma oscillations similar to depressed patients. Although the exact mechanism underlying abnormalities in gamma oscillations in FSL rats needs to be elucidated, it is anticipated to reflect perturbations in the combined networks of pyramidal neurons and GABA interneurons. The experiments were approved by the local ethical committee for animal research in Solna, Sweden. References 1. Liu T-Y. et al. (2012) Different patterns of abnormal gamma oscillatory activity in unipolar and bipolar disorder patients during an implicit emotion task.

Neuropsychologia 50: 1514-1520. 2. Shin Y-W. et al. (2011) Gamma oscillation in schizophrenia. Psychiatry Investig. 8: 288-296. 3. Homayoun H. and Moghaddam B. (2007) NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J. Neurosci. 27:11496-11500.

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Poster

709. Mood Disorders: Animal Models I

Location: Halls A-C

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Program#/Poster#: 709.02/W35

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Antidepressant actions of nucleus accumbens high-frequency stimulation in a preclinical model of treatment resistant depression

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Abstract: The mechanism of antidepressant action of deep brain stimulation (DBS) therapy for individuals suffering treatment resistant depression (TRD) is yet to be fully understood. TRD is often characterized by lower psychomotor activity, alterations in carbohydrate consumption, reduced motivation to engage in rewarding activities, passive coping during exposure to stress and decreased positive affect. Effective antidepressant therapies aim to reverse these behaviors. Although DBS has been demonstrated to be effective for patients who are unresponsive to other treatments, our capacity to optimize efficacy and restrict unwanted side effects remains limited. Mesolimbic structures such as the nucleus accumbens (NAc) are implicated in the antidepressant response and have been trialled as therapeutic targets for DBS. This study investigated the antidepressant effects of NAc DBS in an animal model of TRD. TRD was induced in male Wistar rats through chronic administration of the endogenous stress hormone adrenocorticotropic hormone (ACTH-(1-24); 100µg/day; 2 weeks), coupled with social isolation (3 weeks). Rats were divided into 4 groups: TRD-DBS, TRD-Sham, TRD, and Control. Bipolar twisted electrodes were implanted bilaterally into the NAc for TRD-DBS and TRD-Sham groups. After recovery, 1mL injections were administered daily (i.p. ACTH 1-24 or 0.9% saline). Back

mounted DBS devices were installed and activated following one week of injections. Activity counts were obtained for all rats using infrared beam motion detectors throughout the experiment (1 week of baseline; 2 weeks of treatment). A sucrose preference test was run each week and forced swim test (FST) andopen field tests (OFT) were conducted following 2 weeks of ACTH or saline treatment as models of depression and anxiety. Following the final forced swim test, animals were euthanized and brains removed and frozen on dry ice. There was a significant increase in basal home cage activity for DBS treated rats compared to other groups. Increased active coping behaviours and decreased immobility were also observed during the forced swim test for the TRD-DBS group. In contrast, and validating the results of the FST, no activity differences were observed in the OFT. Interestingly, an increase in sucrose consumption was observed for ACTH treated (TRD) groups not receiving DBS, suggesting a compensatory upregulation of carbohydrate craving had occurred as a result of HPA-axis stimulation. This was not observed in TRD-DBS and Control groups. These data suggest that NAc DBS has antidepressant effects but not anti-anxiety effects in this animal model of TRD.

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Aarhus University

Aarhus University Hospital

Title: The effect of chronic mild stress on pyramidal cell number in four subfields of the rat hippocampus

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[1]Ctr. of Functionally Integrative Neurosci., [2]Stereology and EM Lab., [3]PET centre, Aarhus Univ. Hosp., Aarhus, Denmark; [4]Zoophysiology, Aarhus Univ., Aarhus, Denmark; [5]Dept. of clinical medicine, Aarhus Univ., Risskov, Denmark

Abstract: Depression is known to cause atrophy in the hippocampus. Clinical studies have shown a marked decrease in hippocampal volume in humans following severe and repeated episodes of depression, while animal models of depression have been used to prove a reduction in hippocampal neurogenesis. Reduced neurogenesis alone does not explain the extent of atrophy in human depressives, but seems to play an important role in the pathology of depression since several types of antidepressant treatments work, at least in part, through stimulation of neurogenesis. By investigating the pyramidal cell layers in the hippocampus, this study might elucidate the effect of hippocampal neurogenesis and/or neurotoxicity beyond the dentate gyrus. Chronic mild stress (CMS) is a thoroughly validated rodent model of depression. Chronic exposure to a series of mild unpredictable stressors induces several depressive-like symptoms in most rats. Among these symptoms is a reduced sensitivity to reward (anhedonia) as assessed by a reduction in sucrose consumption. The symptoms are partly reversible by antidepressant treatment, further supporting the validity of the model. However, in approximately 30% of the rats, CMS does not reduce reward sensitivity. This group is thought to be stress resilient. In this study, design-based stereology is used to investigate possible changes in hippocampal morphology in three groups of rats: CMS sensitive (Anhedonic), CMS resistant (Resilient), and a non-stressed control group. Using the optical fractionator and the Cavalieri estimator respectively, the neuron number and volume is estimated in four subdivisions of the cornu ammonis (CA): Ventral CA1, dorsal CA1, ventral CA2+3, and dorsal CA2+3. Preliminary data indicates no significant differences in cell number or volume between anhedonic and control animals in any of the four subfields. The resilient group stood out however, by having approximately 20% more neurons in the ventral CA1 compared to the other groups. A previous study from our lab found a cell loss in the dentate gyrus in the anhedonic groups, and the absence of any neuronal- or volume loss in this study suggests a differential sensitivity of hippocampal cells to CMS. The increased neuronal number in the resilient group supports the idea that stress resilience is not a lack of response to stress but an active response.

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: MEXT Grant Ibaraki University Cooperation between Agriculture and Medical Science (IUCAM)

Title: Effects of chronic mild social defeat stress on characteristics of behaviors and serum components in mice

Authors: *T. GOTO[1,2], Y. KUBOTA[1], Y. TANAKA[1], W. IIO[1], A. TOYODA[1,2,3];
[1]Ibaraki Univ., Ibaraki, Japan; [2]Ibaraki Univ. Cooperation between Agr. and Med. Sci. (IUCAM), Ibaraki, Japan; [3]Tokyo Univ. of Agr. and Technol., Tokyo, Japan

Abstract: Characterization of animal models of psychiatric disorders including depression is important for understanding pathological features in humans. We made and analyzed a model mouse using a chronic mild social defeat stress (CSDS) paradigm which is a modified version of the standard CSDS protocol reported by Golden et al. (Nature Protocols, 2011). In this study, C57BL/6J (B6) male mice were exposed to CSDS using aggressors of ICR male mice. Body weight, food intake, and water intake of B6 were monitored for the experimental periods consisting of 10 days of CSDS paradigm and one month for recovery from CSDS. Behaviors and serum biochemical components of B6 were analyzed at two stages which are immediately after the CSDS and one month after the CSDS. Body weight and food intake of the socially stressed mice were significantly higher than those of non-stressed control mice at the CSDS period, and then these differences were sustained until one month after the CSDS. Water intake of the socially stressed mice was more than twice as much as that of control mice at the only stress period. The socially stressed mice showed defeated behaviors and significantly social avoidance to unfamiliar ICR mice in social interaction test and also a trend of anxiety-like behavior in elevated-plus maze test than control mice at immediately after the CSDS. However, this social avoidance of stressed mice was not sustained until one month after the CSDS. In addition, serum albumin and blood urea nitrogen levels of the defeated mice were significantly lower than control mice at immediately after the CSDS, while these features were disappeared at one month after the CSDS. These results suggest that our model mice using a mild CSDS may show the

similar victims of people suffering from atypical depression, which have a good appetite and rise in body weight.

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

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Title: Perinatal photoperiod affects the serotonergic system

Authors: *N. H. GREEN[1], C. JACKSON[2], D. MCMAHON[2];
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Abstract: Depression and anxiety disorders are significant problems for human health and are thought to involve alterations in brain serotonin signaling. Our laboratory has recently demonstrated that the circadian photoperiod experienced during perinatal development has enduring effects on depression and anxiety related behaviors and on serotonergic neuronal function. Dorsal raphe neurons are acutely modulated by melatonin and mice developed on different seasonal photoperiods are presumed to have different amounts of melatonergic signaling during development due to the different duration of the dark period. We hypothesize that perinatal light cycles exert their enduring influence on depression and anxiety behaviors in part through developmental melatonergic programming of raphe serotonergic neuronal function. We have developed C3Hf+/+ mice, on an equinox (12:12), long (16:8) or short (8:16) photoperiods to determine the effect these developmental light cycles have on the serotonergic system. Our results demonstrate that perinatal photoperiod significantly affects serotonergic neuronal firing rate, 5-HT and its metabolite concentrations, noradrenaline concentrations, expression of key serotonergic genes and depression and anxiety related behaviors during young adulthood. We have also observed that the changes in serotonergic firing rate, monoamine concentrations and affective behaviors are negated in melatonin 1 receptor knockout mice

suggesting melatonin signaling plays an integral role in the developmental photoperiodic programming of multiple aspects of the 5-HT system. These experiments will establish a novel model for exploration of how a pervasive environmental signal, the light dark cycle, may influence the development and long-term function of brain serotonergic neurons and affective behaviors.

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: JSPS KAKENHI Grant 22791126

MEXT SRPBS Grant

Title: Essential role of hippocampal SIRT1 in behavioral response to stress in mice

Authors: ***S. UCHIDA**, N. ABE-HIGUCHI, H. YAMAGATA, T. HOBARA, F. HIGUCHI, K. HARA, A. KOBAYASHI, Y. WATANABE;
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Abstract: Growing evidence supports the hypothesis that epigenetic mechanisms, which control chromatin structure and function, mediate changes in gene expression required for synaptic plasticity and behavior. Recent reports have suggested that epigenetic gene regulation via Class I and II histone deacetylases (HDACs) can trigger the development of stress vulnerability and contribute to behavioral responses to chronic stress and antidepressants in rodents. Class III HDACs, also known as sirtuins, are a family of oxidized nicotinamide adenine nucleotide (NAD+)-dependent enzymes that regulate cellular functions through deacetylation of various protein targets. However, the involvement of sirtuins in the pathophysiology of mood disorders has not been fully elucidated. The aim of this study is to clarify the role of sirtuins on depression-like behavior. We first measured the mRNA levels of sirtuins in the hippocampus of stressed BALB/c mice, which were used as an animal model of depression (Uchida et al., Neuron 2011), by quantitative real-time PCR. We found that the mRNA level of one sirtuin, SIRT1, was significantly lower in the hippocampus of stressed mice than in non-stressed controls. We then

investigated the effect of sirtinol, a sirtuin inhibitor, on depression-like behavior. Intra-hippocampal injection of sirtinol led to increased depression-like behaviors. We also examined the effect of gain-of-function SIRT1 on depression-like behavior. To do this, we overexpressed the SIRT1 protein in the hippocampus of mice using an *in vivo* gene delivery system. We found that mice overexpressing SIRT1 in the hippocampus did not develop depression-like behaviors after chronic stress exposure. These results suggest that hippocampal SIRT1 is critically involved in stress susceptibility and resilience. We further examined the neurobiological role of SIRT1 and found that stress-induced reduction of spine density in hippocampal neurons was prevented by treatment with a SIRT1 activator. Conversely, downregulation of SIRT1 function by intra-hippocampal injection of sirtinol reduced the spine density. Thus, these data suggest that the SIRT1 is involved in structural plasticity in response to chronic stress. From these studies, we conclude that dysfunction of hippocampal SIRT1 might be associated with depression-like behavior in mice through its effects on stress-induced neuronal plasticity.

Disclosures: S. Uchida: None. **N. Abe-Higuchi:** None. **H. Yamagata:** None. **T. Hobara:** None. **F. Higuchi:** None. **Y. Watanabe:** None. **K. Hara:** None. **A. Kobayashi:** None.

Poster

709. Mood Disorders: Animal Models I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 709.07/X4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Neuron-Eranet Project POSEIDON

Fondazione CARIPLO (n. 2012-0503)

Title: Ankyrin-3: Role in stress response and implications for mood disorders

Authors: *A. LUONI[1], R. MASSART[2], V. NIERATSCHKER[3,4], A. CATTANEO[5,6], F. CIRULLI[7], C. M. PARIANTE[5], M. SZYF[2], M. A. RIVA[1];
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Ricovero e Cura a Carattere Scientifico San Giovanni di Dio, Fatebenefratelli Ctr., Brescia, Italy; [7]Dept. of Cell Biol. and Neurosciences, Inst. Superiore di Sanità, Rome, Italy

Abstract: It is well established that the risk to develop mood disorders may increase after exposure to stressful events. In particular, early life represents a critical period during which the nervous system is particularly susceptible to environmental stimuli and adverse events perceived during this critical time may alter the correct trajectory of several systems, thus leading to long-lasting changes in neuronal function. Several animal models and human studies suggest that one of the mechanisms through which the exposure to stress early in life could affect the lifelong phenotypes is epigenetic regulation of gene expression involving, among others, changes in DNA methylation. Taking advantage of an European consortium named POSEIDON, (Pre-, peri- and postnatal stress in human and non-human offspring), we used an unbiased genome-wide and cross-species approach in order to identify genes whose expression may be persistently affected by exposure to stress early in life, through changes in the methylation of their promoters. Among these genes, an interesting candidate is Ankyrin 3 (Ank3), a scaffolding protein involved in cellular trafficking of several molecules such as receptors and channels, which has a strong genetic association for psychiatric disorders. We found that the methylation status of Ank3 is affected in adult rats that were exposed to gestational stress as well as in the brain and T cells from monkeys exposed to maternal deprivation. We thus deepened the analyses of Ank3 modulation in different paradigm of exposure to stress. In line with the changes in methylation, the expression of Ank3 is significantly reduced in the hippocampus and prefrontal cortex of rats that were exposed to stress during the last week of gestation. Moreover, we found that animals that develop anhedonia as a consequence of chronic mild stress exposure have a reduced Ank3 gene expression in the prefrontal cortex. Last, using an in-vitro system of human hippocampal stem cells, we found that treatment with cortisol produces a long lasting decrease of Ank3 expression. This finding suggests a contribution of glucocorticoids in the changes brought about by prenatal stress. In summary, we suggest that Ank3 may represent an important link between stress exposure and the development of psychopathology, through alterations in neuronal circuits relevant for mood regulation and cognition.

Disclosures: **A. Luoni:** None. **R. Massart:** None. **V. Nieratschker:** None. **A. Cattaneo:** None. **F. Cirulli:** None. **C.M. Pariante:** None. **M. Szyf:** None. **M.A. Riva:** 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.; Bristol-Myers Squibb, Sunovion. F. Consulting Fees (e.g., advisory boards); Bristol-Myers Squibb, Dainippon Sumitomo Pharma, Eli Lilly, Roche, Servier, Sunovion.

Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH 1R01MH090264

J&J/IMHRO Rising Star Translational Research Award

NARSAD Young Investigator Award

Irma T. Hirschl/Monique Weill-Caulier Trust Research Award

Title: Bone marrow derived leukocytes underlie susceptibility and resilience to social stress

Authors: *G. E. HODES[1], M. L. PFAU[1], M. C. LEBOEUF[2], S. A. GOLDEN[1], D. J. CHRISTOFFEL[1], M. HESHMATI[1], H. ALEYASIN[1], E. H. F. WONG[3], C. A. BOLAÑOS-GUZMÁN[4], M. MERAD[2], S. J. RUSSO[1];
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Abstract: Repeated social defeat stress (RSDS) is a physical and emotional, ethologically relevant animal model of stress related disorders, such as major depressive disorder (MDD) and posttraumatic stress disorder (PTSD). In this model an experimental mouse (C57BL/6J) is exposed to daily bouts of physical interaction with a larger, more aggressive, retired breeder (CD-1) in the aggressors home cage. After exposure to RSDS some animals termed susceptible show a spectrum of depression-like behavior, whereas resilient animals behave more akin to controls. We have found that peripheral immune dysregulation predicts which animals show susceptibility to RSDS. Additionally, this peripheral immune dysregulation is both necessary and sufficient to induce social avoidance behavior. Bone marrow transplants (BMT) from susceptible donors lead to increased numbers of circulating leukocytes in naïve hosts prior to stress and increased social avoidance behavior after a sub threshold-stress. Additionally, animals that received BMTs from IL-6-/- mice or pretreatment with IL-6 monoclonal antibodies to inhibit peripheral IL-6 display a resilient phenotype following 10 days of RSDS. Furthermore, the peripheral immune system also contributes to stress sensitivity in an emotional/sensory social stress model known as “witness” defeat. In this model, experimental animals witness another animal undergo RSDS. They are housed repeatedly in partitioned cage with a novel aggressor and have sensory, but no physical, contact with the aggressor. BMT from a donor susceptible to RSDS induces greater social avoidance behavior in animals that witnessed RSDS. Additionally,

BMT from an IL-6-/- donor blocked the development of avoidance behavior after witnessing defeat. Together these studies demonstrate a functional role for the peripheral immune system in the development of stress related behavior. These studies provide a basis to explore novel treatment options that act in the periphery for stress related disorders such as MDD and PTSD.

Disclosures: G.E. Hodes: None. **M.L. Pfau:** None. **M.C. Leboeuf:** None. **S.A. Golden:** None. **D.J. Christoffel:** None. **M. Heshmati:** None. **H. Aleyasin:** None. **M. Merad:** None. **S.J. Russo:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Janssen Pharmaceuticals. **C.A. Bolaños-Guzmán:** None. **E.H.F. Wong:** A. Employment/Salary (full or part-time):; Astrazeneca.

Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH

NIDA

Title: Adolescent social stress induces sex-specific effects on cocaine-elicited and anxiety-like behaviors in the adult mouse

Authors: *D. M. WALKER[1], M. A. DOYLE[1], E. J. HARRIGAN[1], H. M. CATES[1], G. E. HODES[1], P. J. KENNEDY[2], D. FERGUSON[3], B. LABONTE[1], H. SUN[1], J. RABKIN[1], E. J. NESTLER[1];
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Abstract: Adolescence is a period of great change in the brain and periphery. Specific stressors during this time cause long-term behavioral changes in adulthood suggesting that adolescence is a critical period of development. The mesolimbic dopamine system is sensitive to stressors during this time and social isolation during adolescence increases preference for drugs of abuse and altered cellular physiology throughout the reward circuitry in male rodents. However, few

studies have explored if there are sex differences in the mesolimbic dopaminergic pathway in response to stressors. The processes regulating adolescent development are sexually dimorphic and there are extensive sex differences in stress responses. Additionally, there are marked differences between the sexes in their sensitivity and responses to drugs of abuse. Here, we sought to identify long-term changes in cocaine-elicited and anxiety-like behaviors in males and females exposed to adolescent social isolation stress. Male and female mice were socially isolated or group housed from postnatal day (P) 22 - P42 when isolated animals were rehoused until adulthood (P70- P90). Adult animals were split into 2 groups to investigate the following: 1) behavioral responses to cocaine as indicated by conditioned place preference (CPP), and 2) behavioral testing for anxiety and sexually dimorphic behaviors (elevated plus maze [EPM] and marble burying). Baseline sex differences in preference for cocaine were observed, with males showing a greater preference for cocaine than females. However, social isolation caused opposite effects in males and females, with cocaine preference increased in males and decreased in females, suggesting that social stress during adolescence results in sex-specific effects on the mesolimbic dopamine system and cocaine reward. We observed baseline sex differences in anxiety-like behaviors, with males showing increased marble burying and decreased time in the open arms of an EPM compared to females. Social isolation in adolescence decreased marble burying in both sexes, but had no effect in the EPM, suggesting that adolescent social stress may have subtle effects on anxiety behavior in adulthood. Taken together, these data suggest that the adolescent transition is a sensitive period for social stress that results in long-term sex-specific behavioral changes in adult mice. Current studies are focused on gene expression changes throughout the reward circuitry in response to cocaine after adolescent social isolation stress. Additionally, we are investigating potential epigenetic mechanisms that underlie these long-term alterations in the adult brain.

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Poster

709. Mood Disorders: Animal Models I

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Program#/Poster#: 709.10/X7

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Identification of novel circuits in the nucleus accumbens involving somatostatin interneurons and their role in cocaine induced plasticity

Authors: *E. A. RIBEIRO[1], J. KOO[2], B. JUAREZ[2], J. SCARPA[1], J. FENG[2], J. RABKIN[1], H. SUN[2], D. WALKER[2], H. CATES[2], M. DOYLE[2], E. MOUZON[2], M. HAN[2], E. J. NESTLER[2]; [1]Neurosci. (MD/PhD), [2]Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: The medial ganglionic eminence (MGE) is a transient structure in the developing brain that gives rise to the majority of striatal interneurons. Numerous reports have appeared over the past several years that MGE transplantation into the adult brain can induce plasticity in a variety of animal models of disease. From epilepsy to Parkinson's disease and neuropathic pain, MGE transplantation has been shown to modify the behavioral endpoints used in each model to assess therapeutic efficacy. However, little evidence has been gathered to provide a mechanistic interpretation of MGE-induced plasticity. In an effort to understand the basic principles underlying this phenomenon, we first characterized the primary synaptic inputs onto somatostatin interneurons in the nucleus accumbens (NAc). We next studied the effect of transplanted fetal MGE cells in cocaine action, based on the notion that the molecular, cellular, and circuit mechanisms that underlie cocaine's behavioral effects are well known. We then used FAC sorting to collect RNA from D1-expressing medium spiny neurons as well as from transplanted cells in the NAc after chronic cocaine exposure. We found that MGE transplantation into NAc significantly decreased behavioral responses to cocaine. We are now studying how MGE transplantation affects the predicted molecular (immunohistochemistry, RNA-seq), electrophysiological, and epigenetic (qChIP, ChIP-seq) changes that typically occur in these mice after repeated cocaine administration. By combining molecular and behavioral analyses we are using bioinformatics approaches to begin to understand how MGE transplantation induces plasticity in the adult brain. This project will thus contribute to the fields of addiction and stem cell transplantation research to ultimately gain a deeper understanding of the fundamental molecular, electrophysiological, and epigenetic mechanisms of neuroplasticity. Supported by NIDA

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH R01 MH099378-01

NARSAD Young Investigator Award from the Brain and Behavior Research Foundation YIA18996

Louisiana Board of Regents Fellowship

Title: GluN2B-containing NMDA receptors regulate behavioral despair and the synaptic changes underlying ketamine's rapid antidepressant actions

Authors: *O. H. MILLER, L. YANG, B. J. HALL;
Tulane University, Neurosci. Program, New Orleans, LA

Abstract: A single sub-anesthetic dose of ketamine, a non-competitive NMDA receptor (NMDAR) antagonist, has rapid and sustained antidepressant effects in human patients. In preclinical rodent models ketamine increases protein synthesis, leading to enhanced excitatory synaptic drive in the medial prefrontal cortex (mPFC). We have previously demonstrated that signaling via GluN2B subunit-containing NMDARs suppresses protein synthesis in cortical neurons under basal levels of activity. We therefore hypothesized that genetic removal of GluN2B from principal cortical neurons (2BΔCtx mice) would increase translation and mimic the actions of ketamine. In acute brain slices from 2BCtx animals we observed elevated excitatory synaptic drive in mPFC, measured as an increase in the frequency of AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs), compared to littermate controls. Ketamine induced an increase in mEPSC frequency in control animals but this was occluded in 2BΔCtx mice. These data demonstrate that GluN2B-containing NMDARs are critically involved in regulating the rapid synaptic changes induced by ketamine. We also show that suppressing tonic activation of GluN2B-containing NMDARs *in vivo*, augments excitatory drive in mPFC circuits, and reduces despair behavior. Together these data provide evidence supporting a role for ambient glutamate and activation of GluN2B-containing NMDARs in the etiology of depression.

Disclosures: O.H. Miller: None. **L. Yang:** None. **B.J. Hall:** None.

Poster

709. Mood Disorders: Animal Models I

Location: Halls A-C

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Regulation of clock gene expression in the chronic mild stress model: Modulatory activity of the novel drug lurasidone

Authors: *F. CALABRESE[1], E. SAVINO[1], A. ROSSETTI[1], M. PAPP[2], R. MOLTENI[1], M. RIVA[1];
[1]Dept. of Pharmacol. and Biomolecular Sciences, Univ. of Milan, Milan, Italy; [2]Inst. of Pharmacology, Polish Acad. of Sci., Krakow, Poland

Abstract: Disruptions in biological rhythms are known to be associated with mood disorders. This has led to hypothesize that abnormalities in the molecular clock may contribute to the development of these disorders and normalization of these changes may be important for therapeutic efficacy. The cellular clock is a transcriptional-translational feedback loop involving a number of different genes that may possess separate functions in circadian rhythms and mood regulation. While this machinery has been extensively characterized in the suprachiasmatic nucleus, little is know on the role exerted by individual clock genes in other brain structures, such as hippocampus and prefrontal cortex, which are important for mood disturbances. In the present study we have employed the chronic mild stress (CMS) model of depression in order to establish if possible alterations in the expression of clock gene machinery in hippocampus and prefrontal cortex. Male Wistar rats were exposed to CMS for 2 weeks and sucrose consumption was used to distinguish between susceptible and non-susceptible animals. Control and CMS-susceptible rats were then randomized to receive chronic vehicle or the novel multi receptor drug lurasidone (3 mg/kg/day) for 5 more weeks, while continuing the stress procedure, in order to evaluate the ability of chronic drug treatment to normalize the phenotype associated with CMS. Our data show that the mRNA levels for Per1 and Per2 are significantly down-regulated in the prefrontal cortex of CMS rats, and this is associated with a slight up-regulation of Bmal1 expression. No changes were found for Clock mRNA levels, whereas a small reduction was found for Cry2 expression. Interestingly, chronic treatment with lurasidone, which per se produced limited changes on clock gene mRNA levels, was able to normalize the molecular changes induced by stress exposure. The modifications of Per1 and Per2 expression after exposure to CMS appear to be anatomically selective, since we did observe similar changes in dorsal or ventral hippocampus. We believe that changes in clock gene expression as a consequence of CMS exposure may contribute to the disturbances associated with mood disorders and may bridge circadian abnormalities with neuronal function in critical brain regions. With this respect, the ability of chronic lurasidone to modulate clock gene expression in association with its ability to normalize the anhedonic phenotype in CMS rats provide further support to its therapeutic properties in ameliorating functions that are deteriorated in patients with major depression and stress-related disorders.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Integrated Research on Neuropsychiatric Disorders carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Gene expression change in the hippocampus of BALB/c mice after chronic ultra-mild stress

Authors: *H. YAMAGATA, T. HOBARA, S. UCHIDA, F. HIGUCHI, N. HIGUCHI, Y. WATANABE;
Dept Neurosci, Yamaguchi Univ. Sch. of Med., Ube Yamaguchi, Japan

Abstract: Chronic stress can lead to changes in hippocampal neurogenesis, which may be involved in the pathophysiology of mood disorders. We previously reported that C57BL/6 mice showed adaptive phenotypes in response to chronic ultramild stress (CUMS), whereas BALB/c mice showed depression-like behaviors in response to the same stress. D epression-like behaviors in stressed BALB/c mice were rescued by chronic imipramine treatment. In this study, we examined how CUMS modulates hippocampal gene expression in BALB/c mice. Hippocampal mRNA was extracted after a 6-week exposure to CUMS, and cDNA was generated for microarray hybridization. Microarray analysis was performed using Agilent Whole Mouse Genome arrays. We found that the expression of approximately 600 candidate genes was altered in response to CUMS. Several of these alterations in gene expression were recovered by chronic imipramine treatment. Therefore, our study findings indicate that these genes might be associated with depression and might have potential antidepressant effects.

Disclosures: **H. Yamagata:** None. **T. Hobara:** None. **S. Uchida:** None. **F. Higuchi:** None. **N. Higuchi:** None. **Y. Watanabe:** None.

Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH09230

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MINH Grant F31 MH095425

Jonhnson & Johnson/IMRO

Title: Lateral habenula projections to a subset of ventral tegmental area neurons rapidly encodes for susceptibility to social defeat stress

Authors: *D. CHAUDHURY[1], H. ZHANG[2], B. JUAREZ[2], A. FRIEDMAN[2], S. KU[2], M.-H. HAN[2];
[1]Pharmacol. and Therapeut., Mount Sinai Sch. Of Med., New York, NY; [2]Mount Sinai Sch. of Med., New York, NY

Abstract: We have recently shown a functional role of ventral tegmental area (VTA) dopamine (DA) neurons in encoding for the depressive (susceptible) phenotype in a chronic social defeat (CSD) model of depression (Chaudhury, Walsh et al., *Nature* 2013). However, the circuit mechanisms by which VTA DA neurons are modulated during the encoding of depression-related behaviours is unknown. The lateral habenula (LHb), a nucleus that functionally integrates signals between limbic forebrain and monoaminergic hindbrain regions, sends robust projections to the VTA. The LHb is known to encode for motivation-, reward- and depression-related behaviours. *In vitro* slice recordings of LHb neurons projecting to VTA (LHb-VTA) show robust increases in activity in stress-susceptible, but not resilient, mice following exposure to CSD stress. To directly correlate the firing dynamics of LHb-VTA neurons in encoding for depression-related behaviours, we optogenetically modulated this circuit in stress-susceptible mice that had previously undergone CSD. Optical silencing of LHb-VTA neurons during a social interaction test, by activating halorhodopsin (NpHR), of previously stress-susceptible mice rapidly induced a resilient phenotype. We are presently investigating the putative mechanism by which LHb inputs modulate the microcircuitry of the VTA. These studies will provide further insight into the role of putative neural circuits modulating the brain reward circuits in encoding

for stress susceptibility. *Supported by NIMH (R01 MH09230, F32 MH096464, and F31 MH095425) and Johnson&Johnson/IMHRO.*

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NWO-ALW grant #819.02.022

Title: Translational psychiatry; findings from a rat model of the interaction between early life adversity and serotonin transporter gene variation

Authors: *R. VAN DER DOELEN[1], W. DESCHAMPS[3], I. A. ARNOLDUSSEN[2], C. D'ANNIBALE[3], F. CALABRESE[4], G. GUIDOTTI[4], D. PEETERS[2], B. GEENEN[2], R. A. WEVERS[2], M. A. RIVA[5], D. ZELENA[6], J. R. HOMBERG[2], T. KOZICZ[2];
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Abstract: Early life stress (ELS) in the form of childhood abuse has been associated with increased risk for the development of psychiatric disorders like major depression. The short allelic variant of the serotonin transporter (5-HTT) promoter-linked polymorphic region (5-HTTLPR) has been shown to modulate the relationship between ELS and psychopathology. To explore the underlying biology of this gene x environment interaction, we have used homozygous and heterozygous 5-HTT knockout rats and their wildtype littermates (5-HTT-/-, 5-HTT+/- and 5-HTT+/+ rats), which were exposed to daily 3 h separations or a control treatment from postnatal day 2 to 14. We found that ELS and 5-HTT genotype interact to affect the hypothalamic-pituitary-adrenal axis by programming the adrenal sensitivity to adrenocorticotropic hormone. Moreover, ELS x 5-HTT genotype interaction affected the central expression of the glucocorticoid and mineralocorticoid receptors, FK506-binding protein 51 and DNA methylation of the promotor regions of the genes encoding for corticotropin-releasing factor and urocortin 1. In addition, we found that ELS and 5-HTT gene variation affect adult

stress coping behaviour in a learned helplessness paradigm. Altogether, we have identified important leads for future investigation which promise to reveal the molecular mechanisms that underlie ELS x 5-HTT genotype interaction effects on behavior and psychopathology.

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Carney Scientific Innovation Fund- Brown Institute for Brain Science

Title: Sex-selective effects of early life stress on depressive outcomes in mice: Continuous home cage video monitoring as a novel method to assess outcomes

Authors: *K. G. BATH[1,2], X. LI[3], T. SERRE[3];
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Abstract: Early life stress profoundly impacts emotional development, and leads to increased risk for later development of affective pathology. Mouse models provide a powerful tool in which to test the effects of various forms of early life stress on behavioral outcomes, as well as the efficacy of novel pharmacological treatments on behavioral normalization. However, current procedures for testing depressive-like pathology in animal models are extremely limited with regard to their efficacy during key developmental periods as well as the ability to assess the complex constellation and permanence of symptoms associated with this form of pathology. We have developed a novel method to continuously and unobtrusively track and quantify home cage behaviors of mice over extended spans of time. We find that female, but not male mice, raised by dams with restricted access to bedding from P4-P11, show a constellation of home cage behaviors that mirror the profile of symptoms associated with depression in humans. This includes a significant reduction in self care, disturbed sleep, altered food intake, and decreased activity compared to age matched unstressed mice. Furthermore, we demonstrate that the behavioral profile of stressed mice can be normalized to control levels within a 24 hour span

following treatment with the recently identified and fast acting anti-depressant ketamine. This monitoring system is freely available, and has the potential to provide a more ethnologically valid measure of depressive-like pathology and assessment of the efficacy of novel pharmacological agents.

Disclosures: K.G. Bath: None. **X. Li:** None. **T. Serre:** None.

Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIDA (R01 DA014133)

Title: Cocaine regulates monoubiquitination of histones H2A and H2B

Authors: *J. RABKIN[1], H. SUN[1], E. S. CALIPARI[1], H. M. CATES[1], E. A. RIBEIRO[1], M. CAHILL[1], R. L. NEVE[2], E. J. NESTLER[1];
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Abstract: Although the etiology of drug addiction is multi-factorial, mounting evidence suggests that drug-induced alterations in gene expression in the brain's reward circuitry contribute to the chronic, relapsing nature of the syndrome. Our group and others have shown that histone post-translational modifications represent one important mechanism by which chronic exposure to cocaine induces these changes. However, histone ubiquitination, despite being known to exist for decades, is less well studied than other histone marks. The dominant form of ubiquitinated histones in the cell are monoubiquitinated H2A and H2B, and both have been shown to regulate transcription in yeast and cultured human cells. However, very little is known about the role of histone ubiquitination in drug addiction. Here, we show that repeated cocaine administration regulates levels of H2A and H2B monoubiquitin in the nucleus accumbens, a key brain reward region, as well as levels of ‘writer’ and ‘eraser’ enzymes that add or remove these ubiquitin marks, respectively. We are currently conducting ChIP-sequencing for H2A and H2B monoubiquitin to identify how these marks are regulated by cocaine both individually and in relation to other histone modifying marks. We are additionally examining the effect of histone monoubiquitination on behavioral responses to cocaine by viral-mediated knockdown or overexpression of histone ubiquitin writer and eraser enzymes. To our knowledge, this is the first

evidence of the role of histone ubiquitination in the pathophysiology of drug addiction and points to a new area of research with potential therapeutic benefits.

Disclosures: J. Rabkin: None. **H. Sun:** None. **E.S. Calipari:** None. **H.M. Cates:** None. **E.A. Ribeiro:** None. **M. Cahill:** None. **R.L. Neve:** None. **E.J. Nestler:** None.

Poster

709. Mood Disorders: Animal Models I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 709.18/X15

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH

HDRF

CIHR

Title: Genome-wide brain gene network alterations in major depressive disorder

Authors: *B. LABONTÉ[1], I. PURUSHOTHAMAN[1], C. TAMMINGA[3], G. TURECKI[4], B. ZHANG[2], L. SHEN[1], E. NESTLER[1];
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Abstract: Major depressive disorder (MDD) is a complex and heterogeneous disorder likely resulting from alterations in several brain regions. Imaging studies in humans with MDD report abnormal activation of several interconnected brain regions involved in mood regulation. These alterations are believed to be accompanied by significant gene expression changes within these brain regions. However, the extent of these transcriptional differences is still unclear and the transcriptional networks engaged across brain regions are poorly understood. This study aims at defining the transcriptional signature associated with MDD and characterizing gene expression networks in several brain regions which are thought to be altered in MDD. Postmortem brain samples from MDD (N=24; 12 males, 12 females) and controls (N=24; 12 males, 12 females) were obtained from the Douglas-Bell Canada Brain Bank. Transcriptional profiles were analyzed in 6 brain regions (medioventral prefrontal cortex (mvPFC; BA25 or Cg25), orbitofrontal cortex (OFC; BA11), dorsolateral PFC (dlPFC; BA8/9), anterior insula, hippocampus (HPC) and nucleus accumbens (NAc)) using RNAseq (50bp paired end). Differential analysis was

performed with DESeq and gene expression networks were identified by means of a whole gene co-expression network analysis (WGCNA). Results were validated by NanoString using both the same samples and a second MDD/control postmortem brain cohort obtained from UT southwestern. Our analyses reveal differential expression of several hundreds of genes in every brain region investigated. We found significant differences between males and females, with certain brain regions being more affected in females (BA11) and others in males (Cg25). Several of these differences were confirmed by NanoString in both postmortem brain cohorts. Gene ontology revealed enrichment of differentially expressed genes in functions related to mood regulation and neuronal connectivity. Using WGCNA, we identified, in males and females, several gene co-regulation networks taking place in and between brain regions, the alteration of which may be responsible for the expression of specific depressive symptoms. Our findings suggest that gene expression changes alter the networks taking place in and between key limbic and cortical brain regions. Altering this precise transcriptional balance may disrupt normal brain activity and interfere with the regulation of mood-related behaviors.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH (NIDA) Grant DA008227

Title: Novel role of microtubules in the dysregulation of reward learning by cocaine

Authors: *E. S. CALIPARI, M. E. CAHIL, D. DAMEZ-WERNO, D. M. WALKER, J. A. LANDRY, Y. L. HURD, E. J. NESTLER;
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Abstract: Reward learning and cue-reward pairing for natural reinforcers is robust, and cue presentation following periods of abstinence elicits seeking behavior for the previously paired reward. While drug reinforcers (i.e., cocaine) elicit reward learning that is similar to natural rewards, forced abstinence results in seeking behavior that increases (“incubates”) as the withdrawal period increases. Incubation does not occur for natural rewards and highlights how

drugs of abuse adapt normal learning mechanisms to exert potent control over motivated behaviors. While reward learning has been attributed to the formation of new synaptic connections via changes in dendritic spine morphology, the mechanism by which this process occurs, and how it is dysregulated in addiction, is not understood. Recently it was determined that microtubules are an integral component of dendritic spine stabilization; however, their role *in vivo* has yet to be elucidated. Here we aimed to determine the temporal profile of food (natural reward) and cocaine (drug reward) self-administration and withdrawal on microtubules and dendritic spine plasticity. To do this, we measured the biochemical changes that occur following cocaine (0.8mg/kg/inj; Fixed Ratio-1; 6hr/session; 10 days) or food (Fixed Ratio-5; 2hr/session; 10 days) self-administration at multiple withdrawal time points, some of which result in high levels of cocaine, but not food, seeking. Here we show that following cocaine self-administration microtubules and microtubule associated proteins (MAPs) are not increased after 24-hour withdrawal periods; however, longer withdrawal periods result in increased levels of beta-tubulin and other MAPs in the synaptosomal fraction. Increases in EB3, a MAP that regulates microtubule polymerization, are positively correlated with cocaine intake during self-administration. Further, the increases in MAPs were associated with a cocaine intake-dependent increase in levels of NR2B (an NMDA glutamate receptor subunit), a traditional marker of dendritic spine stability. Additionally, modulating microtubule growth and assembly altered cocaine reward. We hypothesize that, while reward learning for natural rewards leads to the formation of dendritic spines at early withdrawal time points, cocaine increases microtubule entry into spines and enhances concomitant signaling cascades leading to long-term stabilization of spines and enhanced cue-reward associations that incubate over time and drive addiction-related behaviors.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH

HDRF

Title: Gadd45b is a critical mediator of susceptibility in the social defeat model of depression

Authors: *J. KOO[1], J. FENG[1], B. LABONTÉ[1], E. RIBEIRO[1], R. NEVE[2], M. LOBO[3], E. NESTLER[1];
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Abstract: BDNF in nucleus accumbens (NAc) has been implicated in loss of ability to cope with stress in preclinical models of depression (e.g., chronic social defeat stress). However, the downstream molecular targets and cellular site of action of BDNF in NAc are not well investigated. In the current study, we propose a critical role of GADD45b (growth arrest and DNA damage-inducible 45 beta) in the development of susceptibility to social defeat. Our data show that 10 days of social defeat stress induces Gadd45b expression in NAc of susceptible mice, but not of resilient mice. Manipulations that promote susceptibility to social defeat and increase BDNF signaling in NAc, such as direct infusion of BDNF in NAc or optogenetic stimulation of ventral tegmental area (VTA) dopamine nerve terminals in NAc, also upregulate Gadd45b expression. In addition, we found differential effects of cell type-specific manipulations of BDNF signaling on Gadd45b expression in NAc: genetic deletion of the BDNF receptor TrkB in D2-type neurons (D2 TrkB KO) robustly decreases Gadd45b expression in NAc, whereas D1 TrkB KO increases it. These data suggest cell type-specific regulation and function of GADD45b, mainly through BDNF-TrkB signaling in D2-type neurons, in mediating susceptibility to social defeat stress. Finally, since GADD45b has been reported to control DNA methylation, we examined the GADD45b-induced DNA demethylation at the Bdnf IV promoter, which is the most sensitive to neuronal activity. Methylated DNA immunoprecipitation assays showed that chronic social defeat stress decreases levels of 5-methylcytosine particularly at the Bdnf IV promoter in the NAc of susceptible, but not of resilient, mice and that viral-mediated overexpression of GADD45b also decreases DNA methylation at the Bdnf IV promoter in this region. These data suggest that the induction of Gadd45b in susceptible mice reduces DNA methylation of this promoter. Consistent with this hypothesis, we found that GADD45b overexpression induces Bdnf mRNA expression in NAc of naïve animals, indicating a feed-forward loop between Gadd45b and Bdnf induction in the NAc. These findings suggest that social defeat-induced increases in BDNF protein levels in NAc, which are derived in part from the VTA, also occur locally via demethylation of the Bdnf promoter in NAc mediated by social defeat-induced GADD45b. These studies provide new insight into the molecular underpinnings of stress-induced neuroadaptations in the NAc, with the goal of illuminating novel therapeutic targets for depression.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH

HDRF

Title: Circuit-wide transcriptional profiling in a mouse model of depression

Authors: *R. C. BAGOT[1], I. MAZE[2], I. PURUSHOTHAMAN[3], X. LIU[3], H. M. CATES[1], C. J. PEÑA[3], K. N. SCOBIE[3], H. SUN[3], D. DAMEZ-WERNO[3], L. SHEN[3], B. ZHANG[4], E. J. NESTLER[3];
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Abstract: Alterations in nucleus accumbens (NAC) are implicated in the pathophysiology of depression. Chronic social defeat stress (CSDS) produces a depression-like phenotype in mice associated with robust transcriptional alterations in NAC. The NAC receives major excitatory inputs from the medial prefrontal cortex (mPFC), ventral hippocampus (vHIP), and basolateral amygdala (BLA), among other regions, and alterations in this circuitry regulate depression-like behavior. To examine the mechanistic basis of CSDS effects on this network, we performed next-generation RNA-sequencing on NAC, mPFC, vHIP, and BLA tissue from control animals and mice determined to be either susceptible or resilient to CSDS. We employed both differential expression analyses as well as co-expression network analyses to further characterize circuit-wide transcriptional profiles. Inter-region comparisons of global gene expression patterns across the circuit indicate enhanced synchrony of differential expression between NAC and mPFC in mice resilient to CSDS and increased synchrony between mPFC and vHIP in susceptible mice. We constructed circuit-wide weighted gene co-expression networks in control, susceptible and resilient mice and identified modules of genes exhibiting differential connectivity between conditions. Several of these modules also showed significant enrichment of differentially expressed genes in specific brain regions. Guided by our previous findings of contrasting functional adaptations in mPFC and vHIP in resilience, we selected modules enriched for oppositely regulated differential expression in these brain structures. To identify transcriptional "master regulators" of resilient-specific gene expression profiles, we focused further on genes

with high intra-modular connectivity. Viral-mediated over-expression of one potential master regulator, Sdk1, in mPFC increased resilience to CSDS while the same manipulation in vHIP increased susceptibility. Our findings suggest that susceptibility and resilience to CSDS associate with distinct circuit-wide transcriptional profiles. Susceptibility may arise from a general dysregulation of transcriptional programs in this circuit.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH

HDRF

Title: Early life stress alters gene expression and activity patterns in the mesolimbic dopamine system and enhances susceptibility to depression

Authors: ***C. J. PENA**, I. PURUSHOTHAMAN, A. K. FRIEDMAN, R. C. BAGOT, M.-H. HAN, L. SHEN, E. J. NESTLER;
Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Adverse experiences during childhood enhance the risk of psychiatric disorders and poorer physical health in adulthood. However, little is known about the effects of stress interactions from early life and adulthood on gene expression and neuron firing patterns in the mesolimbic dopamine system. We have developed a paradigm in mice whereby early life experiences alter susceptibility to depression-like behavioral abnormalities and are accompanied by broad changes within the brain's reward circuitry. Mice were standard reared or exposed to early life stress (ELS), reared normally during adolescence, and then half of each group underwent chronic social defeat stress in adulthood. Susceptibility to depression-like behaviors was tested, physiological recordings were captured in the ventral tegmental area (VTA), and RNA-sequencing was performed on nucleus accumbens (NAc) and VTA brain samples. Stress in

the postnatal period significantly enhanced susceptibility to social defeat stress in adulthood. Several measures of anhedonia and anxiety were heightened with ELS in combination with social defeat. RNA-sequencing revealed distinct patterns of gene expression in VTA and NAc subsequent to ELS and social defeat. ELS likewise altered the firing rate and other physiological responses in VTA dopamine neurons, suggesting that activity in this area may be an early marker of enhanced susceptibility to a depressive state. Identification of upstream regulators of these altered expression and activity patterns may inform new targets for intervention in early life critical periods.

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Chronic social stress induces mitochondrial DNA mutation and mitochondrial dysfunction in mouse brains

Authors: *K. DUAN[1], X. LIU[1], T. NI[2], J. ZHU[2], Z. LI[1];
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Abstract: Mitochondrial dysfunction and mutations of mitochondrial DNA (mtDNA) are implicated in psychiatric disorders. However, little is known about the effect of chronic social stress on mitochondria. Here, we used the chronic social defeat (CSD) model, a mouse model of social stress, which induces anxiety, depression and learning abnormalities, to investigate the change in brain mitochondria following social stress. Our analysis of mtDNA mutations revealed that CSD caused a moderate increase in the mutation rate of mtDNA selectively in amygdala but not in the hippocampus. Moreover, our mitochondrial function analysis showed that mitochondrial membrane potential (assessed by staining with the mitochondrial membrane potential indicator TMRE) and the activity of cytochrome c oxidase (COX) in the amygdale but not in the hippocampus were reduced in mice subject to CSD. The decrease in COX activity is likely due to less COX expression in the CSD mice, as we detected a lower level of COX subunit I by immunoblotting. These results indicate that chronic social defeat stress undermines

mitochondrial function and reduces the stability of mtDNA. Hence, mitochondrial dysfunction might contribute to social stress-induced behavioral impairment.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: The Rap1 small GTPase network regulates cocaine-mediated dendritic spine morphogenesis and behavioral reward

Authors: ***M. E. CAHILL**[1], H. SUN[1], R. C. BAGOT[1], J. KOO[1], R. NEVE[2], A. GANCARZ[3], G. L. SCHROEDER[3], D. M. DIETZ[3], E. J. NESTLER[1];
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Abstract: Dendritic spines are the sites of most excitatory synapses in the central nervous system and, as recent studies indicate that spines function independently of each other, they are the smallest known information processing units in the brain. Withdrawal from drugs of abuse is known to cause sustained alterations in the density and morphology of dendritic spines in medium spiny neurons (MSNs) of the nucleus accumbens (NAc), a primary reward region. Functional studies support a direct role for altered spine morphogenesis in NAc medium spiny neurons in contributing to addiction-related behavioral plasticity. Members of the Rho subfamily of Ras-like small GTPases are pivotal regulators of spine morphogenesis in numerous neurons types, including MSNs, and guanine nucleotide exchange factors (GEFs) are direct activators of small GTPases. Studies have only recently begun to elucidate the role for GEF-small GTPase networks in reward and addiction processes. In mouse NAc synaptosomal preparations we show that withdrawal from both investigator-administered and self-administered cocaine causes a biphasic alteration in the expression profile of the Rap1 small GTPase. The downstream effectors of Rap1 in NAc MSNs have not been previously characterized, and here we identify a novel role for Rap1 in regulating the activity of an AKT/mammalian target of rapamycin (mTOR) local translation network in spines during cocaine withdrawal. Using a combination of viral-mediated gene transfer and pharmacological manipulations we reveal that altered Rap1-AKT-mTOR signaling controls NAc spine morphogenesis with concomitant effects on cocaine-mediated

behavioral reward. Further, we have employed optogenetic methods to dissect the excitatory inputs into the NAc that regulate Rap1-AKT-mTOR signaling. Current studies are aimed at understanding the transcriptional mechanisms that regulate Rap1 expression during cocaine withdrawal, which will provide a more comprehensive picture of how nuclear and synaptic neuronal compartments coordinate to regulate behavioral reward. Supported by NIDA

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Poster

709. Mood Disorders: Animal Models I

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH092412

Title: Buprenorphine produces antidepressant-like and anxiolytic responses in mice exposed to chronic models of depressive-like behavior

Authors: ***E. FALCON**, R. SWEENEY, R. LEON, J. JOCHEMS, O. BERTON, I. LUCKI; Univ. of Pennsylvania, Philadelphia, PA

Abstract: Kappa opioid receptor (KOR) antagonists are of increasing interest in the search for novel antidepressants. Studies have shown that selective KOR antagonists produce antidepressant and anxiolytic effects in rodents. However, their transition to the clinic is hampered by their long duration of action. Buprenorphine (BPN) is currently used in the treatment of opiate dependence and chronic pain, with actions as a partial mu-opioid receptor agonist and kappa-opioid receptor (KOR) antagonist. We have previously reported that a low dose of BPN (0.25 mg/kg) produces antidepressant-like and anxiolytic responses in the Forced Swim Test (FST) and Novelty-induced Hypophagia (NIH) Test, respectively. In this study, we set out to investigate the effect of BPN treatment in chronic models of depressive-like behavior, like Chronic Mild Stress (CMS) and Chronic Social Defeat Stress (CSDS). In experiment #1, male C57BL/6J mice were exposed daily to three mild stressors for two weeks. CMS-mice exhibited reduced weight gain and decreased sucrose preference after two weeks of stressors when compared to non-stressed mice. Mice were treated with BPN for 7 d before being subjected to several behavioral tests, such as the Light/Dark (LD) test, the Elevated Zero Maze (EZM), and

the FST. Stressed mice treated with BPN exhibited an increase in the time spent in the light side of the LD chamber and in the open arm of the EZM, as well as a decrease in immobility in the FST, when compared to saline-treated mice. In experiment #2, male C57BL/6J mice were subjected to a CSDS paradigm that involved daily 5-min exposures to aggressive CD-1 mice for 10-days, followed by a Social Interaction (SI) Test. Mice that developed social avoidance after the CSDS were treated with saline or BPN (0.25 mg/kg) for 7 d and retested in the SI test. BPN-treated mice showed a significant increase in social interaction when compared to saline-treated mice. These studies demonstrate that BPN for only 7 d is effective in two mouse models of chronic depressive-like behavior and further supports its use as a novel and rapid acting antidepressant.

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Poster

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Title: Female rats are resistant to the depressive behavioral effects induced by early maternal separation stress

Authors: *J. J. DIMATELIS[1], I. M. VERMEULEN[2], K. BUGARITH[2], D. J. STEIN[3], V. A. RUSSELL[2];
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Abstract: Many stress-related psychiatric disorders are more common in women than in men. Sex differences in the response to anti-depressant treatment have also been reported. We aimed to determine how female rats respond to maternal separation (MS) stress and whether the antidepressant effect of light treatment, previously observed in male rats, could be seen in female rats. We also investigated whether the MAPK signal transduction pathway which has been suggested to be associated with stress-induced depression in humans, is altered by MS in female

rats. The MS paradigm (removal of the dam from the litter for 3 h/day from postnatal day (P) 2-14) was used to induce behavioral changes in female rats, some of whom were also treated with chronic constant light for 3 weeks during adolescence. Ultrasonic vocalizations (22 kHz) were recorded and the forced swim test was conducted before (P 65-67) and after light treatment (P98-99) to determine depressive-like behavior. Key proteins in the MAPK signal transduction pathway (MKP-1, p-ERK, total ERK) and the synaptosomal marker (synaptophysin) were measured in the ventral hippocampus. In contrast to male rats, we found that MS decreased the duration of 22 kHz ultrasonic vocalizations on P65 and subsequent light treatment increased the number of 22 kHz calls at P98, in female rats. Also, contrary to male rats, MS decreased the time female rats spent immobile and increased time actively swimming in the forced swim test at P67. MKP-1 and synaptophysin levels remained unchanged in MS and light-treated female rats. Phosphorylated ERK was reduced in MS animals compared to controls and light treatment of MS animals caused a further reduction of p-ERK in the ventral hippocampus of female rats. The results suggest that female rats may be resistant to MS-induced depressive-like behavior and the behavioral effects of MS in females may involve the MAPK signal transduction pathway.

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Poster

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Research Foundation for Opto-Science and Technology

Title: Lentiviral vector system for potent gene expression in serotonergic neurons in rats and mice

Authors: *K. NAGAYASU[1,2], N. NISHITANI[2], N. ASAOKA[2], M. YAMASHIRO[2], H. SHIRAKAWA[2], T. NAKAGAWA[2], S. KASPAROV[3], S. KANEKO[2];
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Abstract: Serotonergic neurons, which originate from the brain stem, project their axons to a number of the brain regions such as cerebral cortex and hippocampus. Serotonergic neurons have been implicated in many brain functions and mental disorders such as depression and anxiety. However, it is not known which serotonergic projection is responsible for each of these many brain functions, partly due to lack of tools to control the function of serotonergic neurons *in vivo*. Although transgenic mice, in which serotonergic neurons can be controlled using genetic tools, have been generated, genetic tools which is applicable to both mice and rats would also be beneficial. In this study, we developed lentiviral vectors which amplify its gene expression by using GAL4-p65 chimeric protein and GAL4 binding element, for potent and specific gene expression in serotonergic neurons. First we used the construct in which transcription of EmGFP-IRES-GAL4-p65 was driven by rat tryptophan hydroxylase 2 (TPH2) promoter and GAL4 binding element. One week after injection of lentiviral vector in the dorsal raphe nucleus, GFP immunoreactivity was detected specifically in TPH-positive serotonergic neurons, although no fluorescence was observed without staining. Next, we shifted the frame of GAL4-p65 to be in-frame to 11th ATG of the IRES and added WPRE downstream to GAL4-p65 to increase gene expression. One week after injection, GFP fluorescence was observed without staining, almost specifically in TPH-positive serotonergic neurons, suggesting that this modified vector is more potent than parental vectors. To apply these vectors not only to rats but also to mice, we have also replaced rat TPH2 promoter with its mouse ortholog. Moreover, using these new vectors we examined the effect of optogenetic stimulation and silencing of 5-HT neurons in mice, and found that specific stimulation of 5-HT neurons induced antidepressant-like effect, although silencing did not have a pro-depressive effect.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R03MH093760

Title: Effects of light on hypothalamic dopaminergic neurons in an animal model of SAD

Authors: *S. P. DEATS[1], L. YAN[2];
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Abstract: Light has profound effects on mood regulation as exemplified in Seasonal Affective Disorder (SAD) and the therapeutic benefits of light therapy. However, the underlying neural pathways through which light regulates mood are not well understood. Our previous work has developed the diurnal grass rat, Arvicanthis niloticus, as an animal model of SAD. Following a housing condition of either 12:12hr Dim Light:Dark (DLD) or 8:16hr Short Photoperiod (SP) that mimics the lower light intensity/duration in the winter, the grass rats showed increased depression-like behavior compared to those housed in 12:12hr Bright Light:Dark (BLD) condition (Leach et al, 2013a,b). To explore the neural substrates underlying the depression-like behaviors, the present study first examined the dopaminergic neurons in the brains of DLD, SP and BLD animals using immunohistochemistry of tyrosine hydroxylase (TH). We found that the number of TH-ir cells in the hypothalamus was significantly lower in the DLD and SP groups as compared to the BLD group, suggesting the hypothalamic dopaminergic neurons are regulated by environmental lighting and a reduction in the number of these neurons are associated with the depression-like behaviors in the grass rat model of SAD. Our previous work has shown that the depression-like behaviors in the DLD animals are associated with attenuated orexinergic signals, and systemic injection of a selective orexin 1 receptor antagonist (SB-334867) provokes depression-like behaviors in BLD animals (Deats et al, 2014). Therefore, in the next experiment, we tested if the difference in the number of TH-ir neurons between the BLD and DLD groups were due to the different activity in their orexinergic pathways. To this end, the number of TH-ir neurons was compared in grass rats housed in BLD treated with 5 daily injections of either SB-334867 or vehicle. We found that the number of TH-ir cells was significantly lower in the SB-334867 treated group, suggesting that inhibiting the orexinergic pathway results in a reduction of TH-ir cells. Taken together, the present study reveals that the hypothalamic dopaminergic neurons are sensitive to daytime light deficiency and are regulated by the orexinergic pathway. The results support the hypothesis that the orexinergic pathway mediates the effects of light on other neuronal systems that collectively contribute to the light-dependent changes in mood.

Disclosures: S.P. Deats: None. **L. Yan:** None.

Poster

709. Mood Disorders: Animal Models I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 709.29/Y2

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Role of p11 in lateral habenula: Regulation of depression-like behavior

Authors: *J.-S. SEO, Y. KIM, P. GREENGARD;
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Abstract: The lateral habenula (LHb) plays a crucial role in emotional adaptation such as seen in stress response and depression. The LHb regulates the activity of monoaminergic neurons and the release of monoaminergic neurotransmitters. Chronic stress induces activation of the LHb, especially the medial part of the LHb. The stress-induced activation of LHb neurons promotes a strong inhibition of dopaminergic and serotonergic neurons. Hyperactivity in the LHb is seen in both rodent models of depression and depression patients. Therefore, the LHb plays an important pathophysiological role in depression. However, little is known about the molecular and cellular mechanisms underlying these processes. Our previous studies identified p11 (also known as S100A10) as a binding protein of several subtypes of serotonin receptors and demonstrated p11 to be an important regulator of depression-like states. However, the expression of and a role for p11 in the lateral habenula have not been shown. Here, we report the expression of p11 in the medial part of the LHb. Depression-like behaviors are observed in mice after either chronic restraint stress-induced or optogenetic stimulation of the LHb. This behavioral phenotype is recapitulated by the manipulation of p11 in the LHb. These results suggest that stimulation of the LHb may mediate depression-like behaviors through p11 pathway.

Disclosures: J. Seo: A. Employment/Salary (full or part-time):; Rockefeller Univ. **Y. Kim:** A. Employment/Salary (full or part-time):; Rockefeller Univ. **P. Greengard:** A. Employment/Salary (full or part-time):; Rockefeller Univ.

Poster

709. Mood Disorders: Animal Models I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 709.30/Y3

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Consejo Nacional de Ciencia y Tecnología , México (104659 CB-EEstrada-Camarena)

Title: Forced swim test modifies the expression of PKA and CREB in the hippocampus and prefrontal cortex of ovariectomized rats

Authors: *E. M. ESTRADA, M. CILIA GARCIA, A. HERNÁNDEZ MELESIO, N. VEGA RIVERA;
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Abstract: Recent studies showed that lost of ovarian function by ovariectomy (OVX) increases the vulnerability to develop depressive-like behaviors in response to acute stress at the same time that decreases the survival of new cells in the hippocampus of the rat. Furthermore, the OVX reduce the dendritic complexity in the hippocampus. These effects have been associated to the deficit of neurotrophins which expression depends of the activation of protein-kinase-A (PKA) and element of response of cyclic adenosine-monophosphate (CREB), among others, in some areas of the brain. It is possible to consider that the decline of ovarian function plus an acute stress event modify the expression of some components of the signaling pathway of PKA-CREB in the rat brain and contribute to develop depressive-like behaviors. Therefore the aim of present work consisted in to evaluate the effect of acute stress induced by forced swim on the protein expression of some components of the signaling pathway of PKA-CREB and Brain derived neurotrophic factor (BDNF) in the hippocampus and prefrontal cortex of OVX rats. Ovariectomized Wistar rats (250-300g) were assigned at random to two groups: 1) stressed (exposed to two forced swim sessions, FS) and 2) non-stressed, both were manipulated in the same form but the control was no subjected to FS sessions. Forty-five minutes after the second session of stress, rats were sacrificed by decapitation and the blood, for corticosterone measure, and the brain were collected for posterior analysis. Corticosterone was measure as an index of stress response by a commercial ELISA kit. In hippocampus and prefrontal cortex tissues was evaluated the expression of PKA, CREB and pCREB by Western-blot analysis while BDNF was evaluated by ELISA commercial KIT. Results showed that FS increased corticosterone ($p<0.001$). In the hippocampus, FS decreases the expression of PKA, CREB and pCREB ($p<0.01$) at the same time that a reduction of BDNF was detected. In contrast, in prefrontal cortex it was not observed changes in the expression of PKA-CREB-pCREB in response to FS exposure. Therefore, forced swim is a potent stressor that reduces the expression of PKA-CREB-pCREB proteins which could contribute to the reduction of BDNF in a region-specific manner in OVX rats.

Disclosures: E.M. Estrada: None. **M. Cilia Garcia:** None. **A. Hernández Melesio:** None. **N. Vega Rivera:** None.

Poster

710. Mood Disorders Animal Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 710.01/Y4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Effect of manganese exposure on rat emotional behavior and brain morphology

Authors: *T. BIKASHVILI[1], L. GELAZONIA[2], I. LAZRISHVILI[2];
[2]Neurotoxicology, [1]I.Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Excessive aggression is attributed to social factors. Along with this, it is generally accepted that aggression and violent behavior may stem from toxic metals. There is evidence that the manifestations of aggressive behavior may also be caused by an excess of manganese data on the correlation between aggressive, violent manifestations and the level of manganese is mainly indirect and sometimes even contradictory. Hence, the effects of 30 day exposure to different doses of MnCl2 (10 and 15 mg/mL in drinking water) on rat aggressive behavior was studied. In this model, two rodents are introduced to each other in the home cage of one of the animals (the resident) and then allowed to interact for a set period of time. 30-day exposure to 10 mg/mL manganese does not stimulate aggressive behavior in rats, while after exposure to the higher dose (15 mg/mL) 37% of initially nonaggressive animals manifested aggressive behavior and 25% attempted it. The data support the hypothesis that excess manganese in the body is one of the immediate causes of enhancement of interspecific predatory aggressive and violent behavior in rats. Statistically significant increase of motor and oriental-searching activity of the exposed groups was shown by open field test. It was expressed in increase of number of lines crossings, rearing and hole reflex. In regard the anxiety or fear indices (frequency of getting to the central part of the open field) manganese intoxication caused a suppression of fear. As it is known brain regions are different by Mn ions accumulation. We found changes in different parts of brain; however, the highest manganese accumulation was seen in hippocampus and in the neocortex. These brain regions play an important role in the regulation of emotional state and motor activity. Histopathological analyzes of brain sections noted two morphologically distinct altered phenotypes of neurons: (1) shrunk sells with indications of apoptosis, their nucleus and cytoplasm are very difficult to distinguish from each other, integrity of neuronal cytoplasm is not disturbed; and (2) swollen cells with indications of necrosis. Pyknotic nucleus, plasma membrane disruption and cytoplasmic vacuoles are observed in these neurons and they are surrounded by activated gliocytes. It must be mentioned, that in the cortex the majority of damaged neurons were of the first type, in subcortical nuclei - the second type.

Disclosures: **T. Bikashvili:** None. **L. Gelazonia:** None. **I. Lazrishvili:** None.

Poster

710. Mood Disorders Animal Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Interdisciplinary PhD Studies "Molecular sciences for medicine" (co-financed by the European Social Fund within the Human Capital Operational Programme).

Title: Decreased phosphorylation of GluA1 in the hippocampus is associated with depressive-like behavior induced by dietary zinc restriction

Authors: U. DOBOSZEWSKA[1], *A. PILC[2], B. SZEWCZYK[1], B. POCHWAT[1], M. HOŁUJ[1], G. NOWAK[1];
[1]Inst. of Pharmacol., Krakow, Poland; [2]Inst. of Pharmacol., 31-343 Krakow, Poland

Abstract: The study's objective: We have previously shown that the up-regulation of hippocampal NMDA receptor (NMDAR) subunits GluN2A and GluN2B is associated with depressive-like behavior induced by a six-week dietary restriction of trace element zinc. Zinc inhibits NMDAR and potentiates AMPA receptor (AMPAR). GluA1, a major subunit of AMPAR, was found to be decreased in hippocampal samples from depressed patients (Duric et al., 2012) and hippocampal deletion of GluA1 impairs expression of behavioral despair (Freudenberg et al., 2013). These findings suggest that GluA1 may be critically involved in the pathophysiology of depression. GluA1 is regulated by phosphorylation at serine sites, S845 and S831. The synaptic and/or the GluN2A-containing NMDA receptors were found to down-regulate GluA1 phosphorylation at S845 (Ai et al., 2011). Our aim was to examine whether dietary zinc deprivation induces changes in the protein level of GluA1 phosphorylated at serine 845 (pS845 GluA1) and whether these alterations are associated with depressive-like behavior. Methods: Male Sprague Dawley rats were fed with a zinc adequate (ZnA) diet, 50 mg Zn/kg or a zinc deficient (ZnD) diet, 3 mg Zn/kg. Following six weeks of the ZnA or ZnD diet, the behavior of rats was examined in the forced swim test (FST), sucrose consumption test or social interaction test. The level of pS845 GluA1 was measured in the hippocampus using western blotting. Results: Following zinc restriction the rats displayed behavioral despair measured as increased immobility time in the FST, anhedonia measured as reduced intake of sucrose solution and a reduction of social behavior. These behavioral changes were associated with decreased expression of pS845 GluA1 protein in the Hp. Conclusions: Our data shows that decreased

phosphorylation of hippocampal GluA1 at serine 845 is linked to the depressive like behavior induced by zinc restriction. Moreover, it demonstrates that neurobiological changes induced by dietary zinc deprivation resemble that of depression, suggesting that the procedure of zinc restriction in rats may represent an animal model of depression.

Disclosures: **U. Doboszewska:** None. **A. Pilc:** None. **B. Szewczyk:** None. **B. Pochwat:** None. **M. Hołuj:** None. **G. Nowak:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Title: Upregulation of CaMKIIβ by HDAC inhibitors might be involved in structural plasticity and behavioral responses in model mouse of depression

Authors: *T. HOBARA, S. UCHIDA, F. HIGUCHI, N. HIGUCHI, H. YAMAGATA, Y. WATANABE;
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Abstract: Recent reports have suggested that epigenetic gene regulations are closely associated with the development of stress vulnerability, and also contribute to behavioral responses to chronic stress and antidepressants. There is evidence suggesting that histone deacetylase (HDAC) inhibitors have antidepressant-like effects in rodents. However, molecular mechanisms of antidepressant actions induced by HDAC inhibitors remain unclear. The purpose of this study is to clarify molecular mechanisms of antidepressant actions by HDAC inhibitors *in vivo* and *in vitro*. First, we confirmed the antidepressant effect of HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) using model mice of depression. We found that subchronic treatment with SAHA reversed the increased depression-related behaviors in mice subjected to chronic stress. In addition, subchronic treatment with SAHA enhanced the expression of calcium/calmodulin-dependent protein kinase (CaMK) IIβ mRNA in the hippocampus of stressed mice. However, subchronic treatment with imipramine, a tricyclic antidepressant , did not affect the expression of CaMKIIβ as well as depression-related behaviors in stressed mice.

These data suggest that SAHA has rapid antidepressant actions, and that the induction of CaMKIIβ by SAHA may contribute to the antidepressant actions. We also found that subchronic treatment with SAHA enhanced adult neurogenesis in the dentate gyrus (DG) of the hippocampus. Our results showed that doublecortin positive immature neurons displayed increased dendritic arborization after subchronic treatment with SAHA or chronic treatment with imipramine. In addition, mice with partially impaired neurogenesis by methylazoxymethanol acetate (MAM) demonstrated depression-related behaviors. Notably, SAHA recovered the increased depression-related behaviors induced by MAM via stimulating maturation of immature neurons. In contrast, full ablation of neurogenesis by Cytosine β-D-arabinofuranoside hydrochloride (AraC) demonstrated sustained depression-related behaviors in spite of co-treatment with SAHA. Furthermore, cell culture experiments showed the induction of CaMKIIβ mRNA and the increased number of differentiated cells by HDAC inhibitors. Importantly, CaMKIIβ knockdown inhibited the induction of cell differentiation by SAHA. Thus our data suggest that the induction of CaMKIIβ by HDAC inhibitors might be involved in structural plasticity and subsequent behavioral responses to chronic stress.

Disclosures: **T. Hobara:** None. **S. Uchida:** None. **F. Higuchi:** None. **N. Higuchi:** None. **H. Yamagata:** None. **Y. Watanabe:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: JSPS KAKENHI (25430079)

JSPS KAKENHI (26117519)

Sakamoto Research Foundation of Psychiatric Diseases

Title: Structural abnormalities of the nodes of Ranvier by exposed repeated stressful events is associated with the onset of major depressive disorder

Authors: *S. MIYATA, S. SHIMIZU, T. TANAKA, M. TOHYAMA;
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Abstract: Major depressive disorder is probably the oldest and still one of the most frequently diagnosed psychiatric illnesses. Major depressive disorder is one of the leading causes of disturbances in emotional, cognitive, autonomic, and endocrine functions, affecting nearly 7% of the population in Japan. According to the large amount of information on depressive diseases that has been accumulated during recent years, patients with major depressive disorder show an enhanced biologic stress-response mechanism, especially a hyperactive hypothalamic-pituitary-adrenal (HPA) axis and high levels of circulating cortisol. Although dysregulation of the HPA axis by chronic stress is indicative of major depressive disorder, the molecular mechanisms and functional changes in the brain underlying depression are largely unknown. In this study, we have developed an animal model of depression by exposing mice to chronic stress. These mice show depression-like symptoms including chronically elevated plasma levels of corticosterone. Furthermore, we have shown that activation of the PDK1-SGK1-NDRG1 cascade in oligodendrocytes may play a key role in upregulating adhesion molecules and increasing the excess arborization of oligodendrocyte processes in the corpus callosum of chronically stressed mice. In this study, we aimed to understand the relationship between the molecular mechanisms underlying the pathogenesis of major depression and the excess arborization of oligodendrocyte processes in the fiber tracts of chronically stressed mice. To do this, we investigated myelin-axon interactions at the nodes and paranodes of Ranvier in the corpus callosum in these mice. Using electron microscopy, we demonstrated that the nodes and paranodes of Ranvier in the corpus callosum were narrower in chronically stressed mice than in control mice. Immunohistochemical analyses of the expression of Caspr and Nav or Kv1.1 and Caspr indicated that the structures of node boundaries were maintained in repeated stress-exposed mice, whereas the paranodes and juxtaparanode boundaries were more diffuse in repeated stress-exposed mice than in control mice. These results indicate that morphological alterations in nodes and paranodes as well as diffuse expression of Caspr and Kv1.1 might affect axonal activities. Thus, chronic stress-induced dysregulation of the nodes of Ranvier is suggested to be closely associated with the development of major depressive disorder.

Disclosures: **S. Miyata:** None. **S. Shimizu:** None. **T. Tanaka:** None. **M. Tohyama:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH093723

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Title: Sex chromosome complement regulates expression of mood-related genes

Authors: *M. L. SENEY[1], K. EKONG[2], Y. DING[2], G. TSENG[2], E. SIBILLE[1];
[1]Dept. of Psychiatry, [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Studies on mood disorders suggest dysfunctions in brain corticolimbic circuits, including altered gamma-aminobutyric acid (GABA) and modulatory (serotonin and dopamine) neurotransmission. Interestingly, sexual dimorphisms in these systems are also reported, and may underlie the heightened female vulnerability to mood disorders. As genetic sex determines gonadal sex, the role of sex chromosomes cannot be investigated individually in humans. Thus, we used the adult Four Core Genotypes (FCG) mice, in which genetic sex and gonadal sex are artificially decoupled, to examine expression of 13 GABA-related genes and 14 serotonin-/dopamine-related genes under chronic stress conditions. Results were analyzed by 3-way ANOVA (genetic sex x gonadal sex x circulating testosterone). A global perspective of gene expression changes was provided by heatmap representation and gene co-expression networks. The main factor influencing expression of GABA-, serotonin-, dopamine-related gene expression in the frontal cortex was sex chromosome complement, with XY mice consistently having lower gene expression compared to XX mice (Calb1, Gat1, Trkb, Htr2c, Adcy5; $p<0.02$ for all comparisons); these results suggest an unexpected pro-disease effect in XY versus XX mice. This effect was partially opposed by gonadal sex and circulating testosterone; overall however, gonadal sex and circulating testosterone exhibited less pronounced, more complex control over gene expression. Across factors, male conditions were associated with a tightly co-expressed set of signal transduction genes. Since GABA, serotonin, and dopamine changes are also observed in other brain disorders, these findings have broader implications for understanding sexual dimorphism in adult psychopathology. The mice in which we examined gene expression had been exposed to chronic mild stress; thus, it is unclear whether the sex chromosome complement effects that we observed are dependent upon chronic stress exposure. To determine whether the sex chromosome complement effects precede chronic stress and whether they are present before the potential interacting factors of adult hormone exposure, we are currently examining expression of the same genes in pre-pubertal FCG mice under no stress conditions. Presence of the same pattern of sex chromosome complement effect on gene expression would suggest that expression of these genes is genetically programmed.

Disclosures: M.L. Seney: None. **K. Ekong:** None. **Y. Ding:** None. **G. Tseng:** None. **E. Sibille:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Israel Science Foundation (ISF 738/11),

National Institute for Psycobiology in Israel (NIPI NO.7-2011-12)

Title: Identifying the most effective component of a novel herbal treatment for depression and anxiety disorders: Neuronal and behavioral effects in a mice model of unpredictable chronic mild stress

Authors: *R. DORON[1,2,3], S. FIDELMAN[4], M. BLONDER[4], M. FRANKO[4], S. ARMOZA[5], M. REHAVI[5];
[1]The Academic Col. Tel Aviv Jaffa/The Open Unive, Tel Aviv- Jaffa, Israel; [2]Educ. & Psychology, The Open Univ., Ranana, Israel; [3]Sch. of Hlth. and Life Sci., Hadassah Col., Jerusalem, Israel; [4]The Academic Col. Tel Aviv Jaffa, Tel Aviv- Jaffa, Israel; [5]Physiol. and Pharmacology, Sackler Fac. of Med., Tel-Aviv Univ., Tel Aviv, Israel

Abstract: In recent years, depression and anxiety disorders have reached epidemic proportions worldwide. Finding adequate treatments for these disorders is of the utmost importance. The long-term most commonly used treatment is selective serotonin reuptake inhibitors (SSRIs), which have been shown to be effective in treating both disorders but have a low success rate and are associated with a wide variety of side effects, including sexual dysfunction and weight changes. These shortcomings have prompted the search for an alternative treatment. Therefore, our research has focused on investigating the anti-depressant and anxiolytic effects of a novel herbal treatment (NHT), which consists of the following components: Crataegus Pinnatifida, Triticum Aestivu, Lilium Brownie and Fructus Zizyphi Jujubae. Our findings have shown that NHT treatment previously exposed to stress has reduced anxiety and depressive-like behaviors in a similar manner to the SSRI escitalopram but without the previously mentioned side effects of SSRIs. NHT has also normalized the stress response and led to the increase of BDNF levels in the hippocampus. The aim of the current research is to further evaluate the beneficial anxiolytic and anti-depressant effects of NHT as a whole and in comparison to each of its four components and to the SSRI escitalopram. For that purpose, mice were exposed to four weeks of unpredictable chronic mild stress procedure (UCMS), followed by a three week treatment with either: NHT (30 mg/kg), Fu-xiao-mai (30 mg/kg), Da-za (30 mg/kg), shan-za (30 mg/kg), Baihe (30 mg/kg), the SSRI escitalopram (15 mg/kg), saline or intact group (not exposed to UCMS and not receiving any treatment). Following treatment, anxiety-like behavior was evaluated using

elevated plus maze , novel open field tests and depressive-like behavior was evaluated using the forced swim test and the tail suspension test. We have found that treatment with either NHT or its component shan-za, significantly reduced anxiety- and depressive-like behaviors in comparison to saline treated mice. No differences were found in locomotor activity between the different groups in the NOF. In addition, SERT levels in the PFC were significantly higher following treatment with the NHT component shan-za and significantly lower in the PFC following exposure to the SSRI escitalopram, both in comparison to the saline group. The conclusions from this study will enable us to better understand the neuronal mechanisms underlying depression and anxiety disorders, and to detect the most efficient ingredient(s) of NHT for the further efficacy and safety evaluation for future use in patients.

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Poster

710. Mood Disorders Animal Models II

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Support: Pritzker Neuropsychiatric Disorders Research Consortium

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Hope for Depression Research Foundation

Title: Fibroblast growth factor 9 is a novel anxiogenic and pro-depressant agent in the hippocampus

Authors: *E. L. AURBACH[1], E. INUI[1], C. A. TURNER[1], M. H. HAGENAUER[1], K. E. PRATER[1], W. E. BUNNEY[2], R. M. MYERS[3], J. D. BARCHAS[4], A. SCHATZBERG[5], S. J. WATSON[1], H. AKIL[1];
[1]MBNI, Univ. of Michigan, Ann Arbor, MI; [2]Univ. of California, Irvine, Irvine, CA; [3]HudsonAlpha Inst. for Biotech., Huntsville, AL; [4]Cornell Univ., New York, NY; [5]Stanford Univ., Palo Alto, CA

Abstract: Recent gene expression profiling experiments have strongly indicated that the fibroblast growth factor (FGF) family is dysregulated in Major Depressive Disorder (MDD). Most widely characterized, FGF2 is downregulated in several regions of the depressed human brain and plays a protective role in rodent models of mood and anxiety disorders. Here, we focused on another member of the FGF family, FGF9, which we have observed to have contrasting patterns of dysregulation, hypothesizing that it works in functional opposition to FGF2. Our previous analyses have demonstrated that FGF9 is consistently upregulated in several regions of the depressed human brain, but little is known about its function in emotional responsivity. In this series of studies, we analyzed FGF9 expression in postmortem brains of patients with MDD and healthy controls using microarray and qRT-PCR, and we observed that hippocampal FGF9 expression was upregulated in MDD brains relative to healthy controls. We next sought to characterize hippocampal FGF9 in rodent models of depression-like behavior. Stress using a chronic social defeat paradigm led to a significant increase in FGF9 mRNA expression by *in situ* hybridization, paralleling the elevations seen in postmortem human brain tissue. Chronic intracerebroventricular administration of FGF9 resulted in elevated anxiety-like behavior in the elevated plus maze and increased depression-like behavior in the forced swim test relative to vehicle-treated controls. In contrast, knocking down FGF9 expression using a lentiviral vector delivered to the dentate gyrus of the hippocampus produced a decrease in anxiety-like behavior on the elevated plus maze relative to animals treated with a non-silencing control virus. Correspondingly, animals administered the knockdown virus expressed significantly less FGF9 mRNA in the dentate gyrus than non-silencing control animals. Collectively, these results suggest that high levels of hippocampal FGF9 mediate vulnerability to mood and anxiety disorders. Future studies will further ascertain whether FGF2 and FGF9 are physiological antagonists and determine whether they work through the same signaling pathways to mediate effects on anxiety- and depressive-like behaviors.

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Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: National Science foundation for Distinguished Young Scholars of China 81225009

Title: Perineuronal nets in prelimbic cortex protect the rats from chronic stress induced depression-like behavior

Authors: *J. SHI, N. CHEN, D. HU, Y.-X. XUE, W.-L. ZHU, L. LU;
Natl. Inst. On Drug Dependence of Peking Univ., Beijing, China

Abstract: Background: It has been showed that dysfunction of GABAergic neurons may participate the pathogenesis of depression-like behavior. Perineuronal nets (PNNs) is a structure which enwraps sub-populations of GABAergic interneurons. Formation of PNNs synchronizes with the maturation of parvalbumin-positive inhibitory interneurons in cortex in young animals, and in adult animals PNNs is critical for the stabilization of inhibitory interneuron network. We investigate the role of PNNs in chronic stress induced depression-like behavior in rats. Methods: Adult rats were exposed to a 21-day chronic unpredictable stress (CUS) and a 14-day intraperitoneally administered of fluoxetine(10mg/kg) were conducted since the second week of CUS. Behavior tests to evaluate the depression-like behavior included sucrose preference and forced swim test. Chondroitinase ABC (ChABC) (0.1U/side) were microinjected into the prelimbic cortex (PrL) to digest the PNNs, and then the rats received a 10-day CUS procedure and test for depression-like behavior. All rats were perfusion for determination of PNNs in PFC after behavior tests. Results: A 21-day CUS procedure induced depression-like behavior and decreased the number of PNNs in PrL. A 14-day systematic administration of fluoxetine reversed CUS procedure-induced depression-like behavior and the decrease of prefrontal PNNs. Interestingly, degradation of PNNs in prefrontal cortex with ChABC did not influence the depression-like behavior, but rendered the rat vulnerable to a subchronic unpredictable stress which did not induce depression-like behavior in control rats. Conclusions: Our findings indicate that PNNs may participate in development of stress induced depression-like behavior.

Disclosures: **J. Shi:** None. **N. Chen:** None. **D. Hu:** None. **Y. Xue:** None. **W. Zhu:** None. **L. Lu:** None.

Poster

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USC Research Foundation (LPR)

Title: Leptin receptor antagonist administration elicits obesity phenotype in the absence of neurobehavioral changes

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Abstract: Ongoing epidemiological studies estimate that greater than 60% of the adult US population may be categorized as either overweight or obese. There is a growing appreciation that the complications of obesity extend to the central nervous system (CNS), including increased risk for development of neuropsychiatric co-morbidities such as depressive illness. Clinical studies indicate that there is an association between obesity and mood disorders and in support of this hypothesis we have previously shown that rats with an obesity/metabolic syndrome phenotype exhibit depressive-like behaviors. A mechanistic mediator that may link obesity and depressive illness is CNS leptin resistance. Accordingly, the aim of the current study was to examine the ability of a pegylated leptin receptor antagonist (PEG-LRA) that inhibits leptin blood-brain barrier (BBB) transport and leads to a leptin-deficient CNS state, to elicit the development of depressive-like behaviors in adult male rats. Daily administration of the PEG-LRA (7mg/kg, ip) produced the expected increases in food intake, body weight, adiposity and plasma leptin levels compared to vehicle-treated control rats. In order to determine whether this obesity phenotype was associated with the development of depressive-like behaviors, we examined behavioral performance in the open field test (OFT), the sucrose preference test and the forced swim test (FST). No significant differences were seen in locomotor activity or total distance traveled in the OFT, or immobility and active behaviors in the FST. In addition, PEG-LRA-treated rats exhibited no differences in sucrose intake or total fluid intake compared to vehicle-treated controls. Interestingly, while plasma cytokines were elevated, histological

analysis did not identify cell infiltration in adipocytes in PEG-LRA-treated rats. These results suggest that while selective inhibition of CNS leptin activity induces an obesity phenotype, decreases in leptin activity alone cannot elicit depressive-like behaviors and support the hypothesis that a variety of endocrine and metabolic changes may be necessary for the development of co-morbid depression in obese individuals.

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Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Hope for Depression Research Foundation

Pritzker Neuropsychiatric Research Consortium

Title: Stress and FGF2 interact to modulate anxiety-like behavior and hippocampal mTOR signaling

Authors: ***E. K. HEBDA-BAUER**, L. A. DOKAS, Q. WEI, S. J. WATSON, H. AKIL; Molecul & Behav Neurosci Inst., Univ. Michigan, ANN ARBOR, MI

Abstract: The fibroblast growth factor (FGF) system and emotional responsiveness are closely interconnected. In the hippocampus (HPC), FGF2 gene expression is decreased in conjunction with anxiety or depression in humans and rats. The FGF system is also reactive to stress and glucocorticoids. We have shown that an increase in FGF2, by direct administration, activates the mTOR signaling pathway [as measured by mTOR mRNA levels and phosphorylation of ribosomal protein S6 (PO4-S6)] in the dorsal HPC of mice. We now ask whether FGF2 also affects anxiety-like behavior under different stress conditions. In the first condition, normal C57Bl/6 (B6) mice were single-housed (SH; i.e., social isolation stress) for 7 or 14 days prior to a single FGF2 injection (200 ng/g; ip) and elevated plus maze (EPM) testing. The data show that B6 mice exhibit a change in behavior from 7 to 14 days of SH. With 7 days of SH, B6 mice show high exploration and low anxiety-like behavior. In contrast, following 14 days of SH, they

show significantly reduced exploration and high anxiety-like behavior. Following 14 days of SH, B6 mice also exhibit higher basal CORT levels in the AM and higher basal ACTH levels in the PM compared to mice SH for 7 days. Immediately following the EPM (and 1 hr after a PBS injection), B6 mice with 14 days of SH also show increased PO4-S6 in the dorsal HPC. Following an acute FGF2 injection, however, B6 mice do not show SH-time dependent differences in anxiety-like behavior, meaning that FGF2 may serve to normalize such behavior. In the second stress condition, mice with a constitutive overexpression of the glucocorticoid receptor (GRov) in forebrain (i.e., a genetic alteration in the stress axis) were tested in the EPM following a single FGF2 injection. GRov mice exhibit increased emotional lability and decreased basal FGF2 gene expression in the HPC that is highly responsive to acute restraint stress. In contrast to B6 mice, acute FGF2 increases anxiety-like behavior of GRov mice when SH for 14 days. Overall, the data show that social isolation stress causes temporal differences in anxiety-like behavior, circulating hormone levels, and mTOR pathway signaling. Under conditions promoting anxiety-like behavior, acute administration of FGF2 is anxiolytic in normal mice but anxiogenic in mice that show emotional lability (i.e., GRov mice). These data also reveal that the effects of FGF2 on anxiety-like behavior are dependent upon environmental conditions and genetic makeup.

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Poster

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Center for Neurogenomics and Cognitive Research (CNCR) partial funding

Title: A prison for the mind: Neuronal plasticity in depressive-like states

Authors: *D. RIGA[1], P. VAN BOKHOVEN[1], J. E. VAN DER HARST[3], T. S. HEISTEK[2], P. VAN NIEROP[1], R. C. VAN DER SCHORS[1], J. A. TIMMERMAN[2], A. W. PIENEMAN[2], Y.

VAN MOURIK[4], A. N. M. SCHOFFELMEER[4], H. D. MANSVELDER[2], W. J. G. HOOGENDIJK[5], A. B. SMIT[1], S. SPIJKER[1];
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Abstract: Major depressive disorder (MDD) is considered to be the second leading cause of disability world-wide, accounting for more lost productivity than any other psychiatric disorder. This is partially attributed to the cognitive decline that accompanies depression, characterized by persistent impairments related to attention, working and episodic memory and executive functions. The debilitating properties of MDD in the cognitive domain pose questions concerning the underlying neurobiological mechanisms and efficacy of current therapies. Wistar rats were subjected to social defeat-induced persistent stress (SDPS) paradigm, in which five defeat encounters are followed by ~3 months of social isolation. During the last 3 weeks of the paradigm, animals were provided with either antidepressant treatment (imipramine) or behavioral therapy (enriched environment). Effects of SDPS and of the two treatment regimes were examined at the behavioral (anhedonia, spatial memory), the electrophysiological (hippocampal long-term potentiation -LTP) and the molecular (hippocampal synaptic proteome) level. SDPS animals displayed a sustained depressive-like state, including reduced reward anticipation, diminished hippocampal-dependent spatial recognition memory and reduced maintenance of hippocampus CA1 LTP. Both antidepressant and behavioral therapies were able to rescue the hippocampal LTP deficit and the observed cognitive impairments. Furthermore, the SDPS-induced depressive-like state was characterized by specific changes of the synaptic proteome of the dorsal hippocampus. We are currently assessing whether targeted interventions that reverse these changes can indeed prevent the depression-associated cognitive deficits and reduced plasticity. Using the SDPS paradigm, an animal model that possesses high face, construct and predictive validity, we can mimic a maintained depressive-like state that models anhedonia, and cognitive decline. Both antidepressant treatment and behavioral therapy seem promising on reversing these effects. The identification of the molecular mechanism by which hippocampal cognitive deficits and limited plasticity develop during this state of enduring depression hold great promise for future treatment strategies.

Disclosures: D. Riga: None. **P. Van Bokhoven:** None. **J.E. Van der Harst:** None. **T.S. Heistek:** None. **P. Van Nierop:** None. **R.C. Van der Schors:** None. **J.A. Timmerman:** None. **A.W. Pieneman:** None. **Y. van Mourik:** None. **A.N.M. Schoffelmeer:** None. **H.D. Mansvelder:** None. **W.J.G. Hoogendijk:** None. **A.B. Smit:** None. **S. Spijker:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Alterations of fluoxetine efficacy in forced swim test behavior between differentially reared male rats

Authors: *C. J. PETERSON[1], D. ARNDT[2], R. TURNER[2], M. E. CAIN[2];
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Abstract: There are multiple contributing factors involved in the development of depression. The environment and serotonin both play major roles in the progression of the disease. Porsolt and colleagues (1977) originally developed the forced swim test (FST) which has become the most widely used assessment for antidepressant-like behavior in rodents (Slattery & Cryan, 2012). While locomotor behavior is generally reduced as a result of antidepressant drugs and environmental enrichment, the effects of environmental enrichment on FST behavior are inconsistent (Bogdanova et al., 2013). The effects of environmental enrichment on FST outcomes range from increased antidepressant-like states to no effect at all. The effects of isolation rearing on FST behaviors are also inconsistent. Isolation has shown to cause both an increase as well as a decrease in behavioral despair. Therefore, the current study focused on the effects of fluoxetine and differential rearing on FST and locomotor behavior. At 21 days of age, male Sprague-Dawley rats arrived at the lab and were randomly assigned to an enriched (EC), standard (SC), or isolated condition (IC). Following 30 days of rearing, the effects of fluoxetine (20 mg/kg, i.p.) were assessed through a 15-minute locomotor test session. In all three conditions (EC, SC, IC), locomotion was attenuated in rats that received fluoxetine. After the locomotor screen, the effects of fluoxetine were measured through a 15-minute pretest, followed by injections of fluoxetine or vehicle 23.5, 5, and 1 hour(s) before a 5-minute test session. There was a significant increase in immobility in all rats from the pretest to the FST. Pretest results revealed that SC rats swam significantly more than IC rats, and EC rats exhibited significantly more climbing than both IC and SC rats, suggesting baseline differences in escape-directed behavior due to differential rearing. The test session revealed no significant differences in immobility between EC, IC, or SC rats receiving fluoxetine compared to their vehicle counterparts. This finding suggests fluoxetine (20 mg/kg, i.p.) did not produce an antidepressant-like effect in enriched, isolated, or standard condition rats. Interestingly, EC-saline rats swam significantly more than their counterparts receiving fluoxetine (20 mg/kg, i.p.). These results suggest that at this dose, fluoxetine was not protective against depressive-like states in the FST, which may be due to the ability of differential rearing, specifically environmental enrichment, to alter the efficacy of fluoxetine.

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Poster

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R01 MH092306

Title: KCNQ3 channel regulation in the VTA mediates social avoidance behavior in response to chronic social defeat stress

Authors: ***A. K. FRIEDMAN**[1], J. J. WALSH[2], B. JUAREZ[2], S. M. KU[2], D. DIETZ[2], M. RIBADENEIRA[3], E. WONG[3], R. NEVE[4], M.-H. HAN[2];
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Abstract: There is an urgent need for mechanistically distinct antidepressant therapies, as less than half of major depressive disorder (MDD) patients achieve full remission with monoamine-based antidepressants. Since maintenance of healthy mental functioning is closely associated with the dopaminergic pathways of the mesolimbic circuit, we explored this circuit for novel therapeutic targets. Specifically, we examined the dopamine (DA) neurons of the ventral tegmental area (VTA) in this reward-circuit. Employing a chronic social defeat stress model of depression, we previously showed an increase in the *in vivo* firing and bursting activity of VTA DA neurons of susceptible, but not resilient mice. Utilizing tyrosine hydroxylase-GFP mice (C57BL/6) to identify VTA DA neurons, we demonstrated that resilient animals homeostatically maintain healthy DA neuron activity through a compensatory upregulation of potassium (K+) channels. In support of our hypothesis that K+ channel upregulation in DA neurons is a mechanism of resilience, we found that selectively blocking KCNQ channels in resilient animals decreased the sustained component of K+ currents, thereby increasing the firing rate of DA neurons. This resulted in making previously resilient mice socially avoidant, highlighting the

importance of these channels. Next, we demonstrated that viral upregulation of KCNQ3 channels in VTA DA of susceptible mice reversed social avoidance. With the goal of therapeutically mimicking the naturally resilient ionic mechanism, we examined a series of pharmacological potentiators of KCNQ channels for antidepressant action. We found that the KCNQ potentiators can rapidly reverse social avoidance, increase sucrose preference and decrease immobility time during the forced swim test. These findings demonstrate that K+ channel currents counteract the pathophysiological hyperactivity of VTA DA neurons and pharmacological potentiation of this naturally occurring resilience mechanism may function as a rapid antidepressant.

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Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NSERC

CIHR

Title: Preclinical model of depression with recurrent episodes: Search for mechanisms of cyclicity

Authors: *K. A. LEBEDEVA[1], E. FENTON[2], R. BOWEN[3], H. CARUNCHO[2], L. KALYNCHUK[4];
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Abstract: Objective: Major depression is a recurrent condition. The existing animal models of depression have not addressed the sensitization that develops over the course of multiple depressive episodes, and therefore, they do not provide means to understand the neurobiological mechanisms by which depression becomes increasingly autonomous of stress. In our recent work, we have proposed the preclinical model of depression with episode recurrence, and investigated the potential neurobiological mechanisms underlying the cyclical nature of depression. Methods: We assessed depression-like behavior in rats through 3 cycles of treatment

with and recovery from the stress hormone corticosterone (CORT). Each cycle of treatment comprised 21 days of CORT injections (10, 20, or 40 mg/kg) followed by 21 days of stress-free recovery. Depression-like behavior was measured in the middle and end of each CORT treatment and at the end of each recovery period using the forced swim test (FST), and sucrose preference test (SPT). Open field test was conducted to measure general locomotor activity of rats. Brain tissue of rats was analyzed for the number and maturation rate of immature neurons in the dentate gyrus. Results: CORT administration produced increasingly greater effects on depression-like behavior in the FST through each cycle of treatment. In the first cycle, CORT increased depression-like behavior after 21 days of treatment, which then normalized after the recovery period. In the second and third cycles however, CORT induced an early manifestation of depression-like behavior after only 10 days of treatment. Furthermore, CORT-treated animals showed increasingly greater anhedonia-like behavior as measured with SPT. No differences were found in general locomotor activity between treatment groups, suggesting no change in rats' exploratory behavior. Finally, CORT also produced physiological alterations indicative of depression: decreased body weight gain (1st cycle) and body weight loss (2nd and 3rd cycles) and accumulative decreases in the number and dendritic complexity of immature granule neurons. Conclusion: These results present a promising model that imitates sensitization to stress that develops over the time of successive depression-like episodes in rodents.

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Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: UNH internal grant

Title: Ultrasonic vocalizations during intermittent swim stress forecast resilience to stress-induced social anxiety

Authors: *R. C. DRUGAN[1], A. JONES[2], N. P. STAFFORD[2];
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Abstract: In the past we have reported that ultrasonic vocalizations (USVs) during intermittent swim stress (ISS) forecast resilience on instrumental learning, behavioral depression, and spatial learning deficits typically observed 24 hours later. Although interesting, these findings are all based on the same context (i.e., water motivated) as the original stressor, so the question of trans-situational generality exists. In the present study, we exposed rats to a baseline social exploration (SE) test with a juvenile. Twenty-four hours later rats were given either 80 trials of ISS or confined control (CC) treatment. One day later all rats were evaluated in the SE anxiety test. ISS exposure did not cause a reduction in SE, but the one rat that did emit USVs during the ISS showed the highest level of SE of any animal tested. This preliminary data demonstrates trans-situational generality of USV-induced resilience in a non-water based anxiety test following ISS exposure.

Disclosures: R.C. Drugan: None. **A. Jones:** None. **N.P. Stafford:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NARSAD Young Investigators Award

Title: Nucleus accumbens projection neuron subtypes mediate depression-related outcomes to social defeat stress

Authors: *T. C. FRANCIS[1], R. CHANDRA[1], D. M. FRIEND[2], E. FINKEL[1], G. DAYRIT[3], J. MIRANDA[4], J. M. BROOKS[1], S. D. IÑIGUEZ[3], P. O'DONNELL[1], A. KRAVITZ[2], M. LOBO[1]; [1]Dept. of Anat. and Neurobio., Univ. of Maryland, Baltimore, Baltimore, MD; [2]Diabetes, Endocrinology, and Obesity Br., Natl. Inst. of Diabetes and Digestive and Kidney Dis., Bethesda, MD; [3]Dept. of Psychology, California State University, San Bernardino, San Bernardino, CA; [4]Univ. Nacional Autónoma de México, Tlalnepantla, Mexico

Abstract: The Nucleus Accumbens (NAc) plays a critical role in reward and motivation. The principle output neurons of the NAc, medium spiny neurons (MSNs), are differentially enriched in Dopamine 1 (D1) and Dopamine 2 (D2) receptors. Altering the activity of these MSN subtypes produces opposite behavioral effects to rewarding and reinforcing stimuli. However, their role in stress has yet to be fully characterized. To examine these outcomes we use chronic

social defeat stress (CSDS), a high-throughput depression model in which mice are exposed to 10 days of CSDS with an aggressive CD-1 retired breeder. Mice susceptible to CSDS display depression-like behaviors including spending significantly less time interacting with a novel mouse and deficits in sucrose preference (SP), a measure of anhedonia, while resilient animals interact similarly to non-defeated mice and display normal SP. We first utilized bacterial artificial chromosome (BAC) transgenic mice expressing GFP in either NAc D1- or D2-MSNs to probe subtype specific electrophysiological outcomes to CSDS. Miniature excitatory post synaptic currents (mEPSCs) in D1-MSNs of susceptible mice were decreased in frequency in comparison to non-defeated animals. In contrast, an increase in frequency in mEPSCs was observed in D2-MSNs from susceptible animals. No changes in mEPSC amplitude were detected. Next, we optogenetically stimulated MSN subtypes of the NAc using BAC transgenic mice, expressing Cre in either D1-MSNs or D2-MSNs, by injecting an Cre-inducible Adeno-Associated Virus (AAV) containing a fast-kinetics channelrhodopsin ChR2(E123A) (ChETA-A). We found that 5 days of repeated 50 Hz 473 nm blue light stimulation of ChETA-A containing D1-MSNs restored normal social interaction and enhanced SP in previously susceptible animals, an effect not observed after stimulating D2-MSNs. We then used a subthreshold social defeat stress (SSDS) paradigm and found that 5 days of repeated 50 Hz stimulation of D2-MSNs prior to SSDS produced social avoidance without changing SP. Using D1-Cre or D2-Cre mice, we pharmacogenetically inhibited NAc MSN subtypes using the Cre-inducible AAV containing the hM4Gi Designer Receptor Exclusively Activated by a Designer Drug (DREADD). Repeated inhibition of D1-MSNs, but not D2-MSNs via an intraperitoneal injection of the specific DREADDs receptor ligand clozapine-N-oxide in resilient animals produced a robust decrease in social interaction and SP. These results indicate that D1- and D2-MSNs have differential roles in the expression of depression-like behavioral outcomes to social defeat stress.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

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Title: Kynurenic acid disrupts stress-induced dopamine response in the rat prefrontal cortex following prolonged reduction of glucocorticoid formation

Authors: *H.-Q. WU, F. M. NOTARANGELO, J. I. KOENIG, R. SCHWARCZ;
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Abstract: Kynurenic acid (KYNA), a metabolite of the kynurenine pathway of tryptophan degradation, is an antagonist of both the NMDA and the alpha-7 nicotinic receptors. Endogenous KYNA modulates extracellular dopamine (DA) levels in the rodent brain in both physiological and pathological conditions. In one recent example, we showed that the well-established, probably advantageous, increase in extracellular DA levels in the medial prefrontal cortex (mPFC) following a restraint stress is abolished in rats after adrenalectomy (ADX), and that this effect is secondary to an abnormal increase in extracellular KYNA (Wu et al., SfN 2013). To investigate the role of reduced glucocorticoids (GS) in this paradigm, we now tested the effect of a GS synthesis inhibitor, metyrapone (MET). To this end, adult male animals were chronically treated with MET (50 mg/kg, p.o. twice at day) for 7 days and then analyzed with microdialysis on the last day of the treatment. On the day before the experiment, microdialysis guide cannulae were implanted over the mPFC. The next morning, microdialysis probes were inserted, and perfusate samples were collected continuously during a 2-hour baseline period before the last MET administration. One hour later, animals were exposed to a 2-hour restraint stress followed by a 4-hour recovery period. Extracellular levels of KYNA and DA were determined in the same dialysates. Analogous to ADX rats, chronically MET-treated animals showed a significant rise in extracellular KYNA levels (156% over baseline) after stress, whereas the normal stress-induced elevation in extracellular DA levels was not observed. In separate animals, 7-day co-administration of dexamethasone (0.2 mg/kg, p.o.), a synthetic GS, with MET reversed the observed biochemical changes, preventing the increase in extracellular KYNA and restoring the normal stress-induced elevation in DA levels (143% over baseline). Furthermore, the stress-induced KYNA elevation observed in chronically MET-treated rats was completely abolished, and the DA increase was re-instated, by co-treatment, on the final day, with BFF816 (30 mg/kg, p.o.), a selective inhibitor of KAT II (the enzyme responsible for functional KYNA synthesis in the brain). KYNA regulation of DA was therefore critical for the results observed in our experiments. Taken together, these findings indicate that a chronic reduction in GS synthesis alters the response of both KYNA and DA to stress in the mPFC, as previously observed in animals after ADX. Pharmacological inhibition of stress-induced KYNA synthesis may provide a novel approach for post-traumatic stress disorder and other afflictions associated with GS dysfunction.

Disclosures: H. Wu: None. **F.M. Notarangelo:** None. **J.I. Koenig:** None. **R. Schwarcz:** None.

Poster

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INP Grant (NC 123240.1)

Title: Deep brain stimulation in thalamic reticular nucleus promotes hippocampal neurogenesis and induces antidepressant-like effect

Authors: *V. M. MAGDALENO-MADRIGAL[1], C. R. PANTÓJA-JIMÉNEZ[1], F. ARREDONDO[2], L. ORTÍZ[2], R. FERNÁNDEZ-MAS[1], S. ALMAZÁN-ALVARADO[1], G. B. RAMÍREZ-RODRIGUEZ[2];
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Abstract: Deep brain stimulation (DBS) has been used as a new treatment for the control of movement disorders, epilepsy, obsessive-compulsive disease, major depression among others. However, the underlying mechanisms of action remain unknown. In the present study we analyzed the effect of DBS in thalamic reticular nucleus (NRT) on hippocampal neurogenesis and behavioral changes. Thirty-one Wistar male rats (280-320 g) were used and stainless steel tripolar electrodes were implanted into the left NRT. Epidural jewelry screws were implanted in the motor cortices (MCx) for EEG recordings and another was connected as a ground in the parietal bone. Stimulation parameters consisted of a 10 min sequence of biphasic square wave pulses at the following levels: frequency, 100 Hz; pulse width, 0.5 ms; and intensity, 200 μA. The DBS in NRT promotes cell proliferation in the subgranular zone of the dentate gyrus; effect that was accompanied with an increase in the number of new neurons-associated to doublecortin. On the other hand, the DBS in NRT induces antidepressant-like effect by showing a significant decrease on immobility-behavior and a significant increase on climbing behavior. Our results suggest that hippocampal neurogenesis induced by DBS in NRT may be a possible mechanism involved in the beneficial effect of DBS in experimental model of neuropsychiatric disorders. Also, provides experimental support for the concept that NRT may be a promising target for focal stimulation to treat the neuropsychiatric disorders.

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Poster

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IMHRO

NARSAD

Title: Optogenetic modulation of the prefrontocortical-dorsal raphe microcircuit bidirectionally biases socioaffective decisions after social defeat

Authors: *C. CHALLIS[1,2], C. MIN[1], S. G. BECK[2,3], O. BERTON[1,2];
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Abstract: Serotonin (5-HT) plays a conserved role in humans and animals in modulating social perception and response to social threats. However, the circuit mechanisms that control social interaction are not well understood. Determining these underlying pathways would provide the groundwork to develop therapeutic interventions that treat socioaffective disorders more precisely. Here, we examined the organization, plasticity and function of the microcircuit formed between 5-HT neurons in the dorsal raphe nucleus (DRN) and top-down projections from the ventromedial prefrontal cortex (vmPFC) in social approach-avoidance decisions. We explored the role of this microcircuit in chronic social defeat stress (CSDS), which results in a long lasting

form of generalized social avoidance that is reversible by antidepressants. Using viral tracing in population specific *Cre*-driver mice we showed that vmPFC projections primarily localize to GABA-rich areas of the DRN, then using optogenetics, whole cell electrophysiology and *cFos* mapping we provide the first direct evidence that vmPFC axons drive synaptic activity and immediate early gene expression in DRN GABA neurons through an AMPA-dependent mechanism. Furthermore, we showed that these GABA neurons locally inhibited 5-HT neurons within the DRN. Next, we optogenetically increased vmPFC input to the DRN during sensory exposure to an aggressor's cues, which produced an enhanced avoidance bias. In contrast, optogenetically decreasing vmPFC drive during CSDS prevented the acquisition of an avoidance phenotype. Finally, we highlight a strategy using *in vivo* recording, microdialysis and optogenetics to characterize the real-time activity of neurons within the vmPFC-DRN microcircuit in social approach and avoidance behaviors. Our results clarify the functional organization of vmPFC-DRN pathways and identify GABAergic neurons as a key cellular element filtering top-down vmPFC influences on affect-regulating 5-HT output.

Disclosures: C. Challis: None. **C. Min:** None. **S.G. Beck:** None. **O. Berton:** None.

Poster

710. Mood Disorders Animal Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 710.20/Y23

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: CNPq

CAPES

UFRGS

UFCSPA

Title: Taurine increases the α2 GABAA receptor subunit and the brain-derived neurotrophic factor (BDNF) mRNA expression in the hippocampus of diabetic rats

Authors: *R. GOMEZ[1,2], G. CALETTI[2], F. B. ALMEIDA[2], T. L. B. DE MELO[2], G. N. CINTRA[2], G. AGNES[2], M. S. NIN[2,3], H. M. T. BARROS[2];
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da Saúde de Porto Alegre, Porto Alegre, Brazil; [3]Ctr. Universitário Metodista do IPA, Porto Alegre, Brazil

Abstract: Taurine, one of the most abundant free amino acids in the central nervous system, modulates a variety of biological functions and acts as an agonist at GABAA receptors. Previous studies showed that taurine decreases depressive-like behaviors in diabetic rats (Caletti, et al., 2012, Amino Acid, 43(4):1525-33). Because changes in the α2 GABAA receptor subunit and in the brain-derived neurotrophic factor (BDNF) are related to mood disorders we studied here the effect of taurine on expression of these two biomarkers in the hippocampus of diabetic rats exposed to the forced swimming rats. Streptozotocin -induced diabetic male adult Wistar rats were matched to control non-diabetic rats and daily administered with saline or 100 mg/kg of taurine (n=10/group), via intraperitoneal. This dose was chose because showed an antidepressant effect in diabetic rats. After 28 days, animals were exposed to the forced swimming test and 30 min later were euthanatized. The hippocampus was removed and the analysis of the relative gene expression of the α2 GABAA receptor subunit and BDNF were performed using reverse transcription combined with real-time quantitative PCR (qPCR) and the $2^{-\Delta\Delta CT}$ method. Our results showed that taurine increases mRNA expression of both the α2 GABAA receptor subunit ($P = 0.016$) and Brain-Derived Neurotrophic Factor (BDNF) ($P = 0.047$) in the hippocampus of STZ rats. Thus, the antidepressant effect of taurine may be explained by its neuromodulatory effect on the GABAergic system and on neuronal growth factors.

Disclosures: R. Gomez: None. **G. Caletti:** None. **F.B. Almeida:** None. **T.L.B. de Melo:** None. **G.N. Cintra:** None. **G. Agnes:** None. **M.S. Nin:** None. **H.M.T. Barros:** None.

Poster

710. Mood Disorders Animal Models II

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Support: Klarman Foundation Grant

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Fullbright Graduate Study Grant

Title: Anxiety level following food restriction predicts hyperactivity in adolescent female mice undergoing activity-based anorexia

Authors: *G. S. WABLE, J.-Y. MIN, Y.-W. CHEN, C. AOKI;
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Abstract: Activity-based anorexia (ABA) is an animal model in use for five decades to identify the biological basis of anorexia nervosa (AN) and AN relapse. When food access is limited to 2 h per day, mice that have been acclimated to a running wheel begin to increase running manifold over baseline, even during the limited hours of food access. Thus ABA captures two salient features of AN - over-exercise and voluntary food restriction. AN is commonly co-morbid with an anxiety disorder (Kaye et al 2004). Yet the anxiety level of animals in ABA has not been reported. We have noted individual differences in the food restriction-evoked hyperactivity (Chowdhury et al. 2013), as some increase their running only minimally (100%), while others increase dramatically (>300%). We ask: Does FR induce hyperactivity, because they are more anxious? If so, is the degree of anxiety correlated with degree of hyperactivity? We measured the level of anxiety in female pubertal mice during wheel acclimation using the open field (OF) test and again during FR phase of ABA using the elevated plus maze test (EPM) in four experimental groups: ABA (access to wheel and restricted food access after wheel acclimation, N = 12), EX (access to wheel and unrestricted access to food, N = 8), FR (no running wheel and restricted access to food per the timeline of the ABA group, N = 8) and CON (ad lib access to food and no wheel, N = 8). We show that the food restriction as well as exercise has varied effects- increase in anxiety in some mice, decrease in anxiety in others while no change in anxiety level in yet other mice. The wheel activity of ABA mice on the second day of food restriction, when most mice do become hyperactive in response to the food restriction, correlates with their anxiety level ($R = -0.83$, $p = 0.01$). In contrast, EX animals did not show a relationship between anxiety level and running activity, suggesting that wheel activity is motivated by anxiety in the presence of food restriction. The degree of vulnerability to ABA is measured in terms of the weight loss and hyperactivity during food restriction and thus we find a close relationship between anxiety and the vulnerability to ABA. This relationship parallels the co-morbidity between anxiety and AN and strengthens the animal model.

Disclosures: G.S. Wable: None. **Y. Chen:** None. **J. Min:** None. **C. Aoki:** None.

Poster

710. Mood Disorders Animal Models II

Location: Halls A-C

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Program#/Poster#: 710.22/Y25

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Differences in antidepressant drug sensitivity in inbred strains in the chick anxiety-depression model

Authors: *S. W. WHITE[1], K. J. SUFKA[2];
[1]Dept. of Psychology, [2]Dept. of Psychology and Pharmacol., Univ. of Mississippi, University, MS

Abstract: Research has identified genetic lines of chicks that show social-separation stress-resiliency (Production Red) and -vulnerability (Black Australorp) in measures of behavioral despair. Antidepressant screenings comparing group means show the resilient Production Red line is sensitive to imipramine but not ketamine and fluoxetine while the vulnerable Black Australorp line is insensitive to imipramine and fluoxetine but is sensitive to ketamine. Both lines were sensitive to maprotiline. However, it is unclear whether individual differences in drug sensitivity exist in chicks within a given strain. Separation-induced distress vocalizations across the anxiety-depression phases were plotted for each chick under each drug dose and compared to the mean for the vehicle control. From this, we sought to identify the percentage of chicks under each drug dose that fell above the 95% confidence interval of the vehicle group in at least two or more consecutive quartiles (15 min each) of the depression-like phase. These analyses revealed patterns of unexpected antidepressant drug effects that illustrate response variability within both strains. These findings suggest the possibility that early developmental factors may alter drug response in these inbred genetic lines in the chick anxiety-depression model.

Disclosures: S.W. White: None. **K.J. Sufka:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Institute for Basic Science(IBS)_EM1405

Title: Stress-induced alterations in neural responses following hindlimb electrical stimulation of rat

Authors: ***S. LEE**[1], H. PARK[1], M.-K. SHIN[2], J. MIN[1], S. LEE[1], Y. LEE[1], E. BAEG[1], Y. LEE[1], M. SUH[1];
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Abstract: Consistent exposures to stress can lead to various illnesses including hypertension, headache, and cardiovascular disorder. In particular, repeated stress causes changes in neuroendocrine regulation and neurotransmitter releasing system resulting abnormal brain function and psychological disease like depression. In addition, stress alters neuronal morphology and population in several brain areas such as hippocampus, amygdala, and prefrontal cortex. The mechanism of the stress-related brain dysfunction, however, has not been clarified at system level. Here, we investigated stress-induced alterations in *in vivo* neural responses while processing peripheral sensory input. Rats were administered with restraint stress 2 hours per day for 3 weeks. After 3 weeks of chronic stress session, we conducted neurohemodynamics imaging study following hindlimb electrical stimulation. The stimulation evoked cerebral blood flow changes and we recorded them with optical recording of intrinsic signal system with 570 nm bandpass filter. This technique can detect perfusion changes based on the absorbance of total hemoglobin volume. We observed that the animal under chronic stress exhibited decreased hemodynamic response in somatosensory cortex upon 5 seconds of hindpaw electrical stimulation (1mA or 2mA, 3Hz, 5ms pulse duration) when it was compared to unstressed group. We also found a reduced cell proliferation of brain cortex in chronic stress group by using western blot analysis. Our results suggest the chronic stress can alter neural responses at systematic level.

Disclosures: S. Lee: None. **H. Park:** None. **M. Shin:** None. **J. Min:** None. **S. Lee:** None. **Y. Lee:** None. **E. Baeg:** None. **Y. Lee:** None. **M. Suh:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant #R01NS045195

Title: The single prolonged stress (SPS) model of posttraumatic stress disorder (PTSD) induces a ptsd-like phenotype in male rats but a depressive-like phenotype in female rats

Authors: *A. POOLEY, A. J. ROBISON, M. S. MAZEI-ROBISON, A. L. EAGLE, S. M. BREEDLOVE, C. L. JORDAN;
Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Men and women respond differently to traumatic stress, but the neurobiological basis for this is not understood. To our knowledge, this is the first study to systematically examine the sex-specific effects of SPS, a validated PTSD animal model, in adult rats. Used to detect disruption of the hypothalamic-pituitary-adrenal (HPA) axis, the dexamethasone (DEX) suppression test indicates that patients with PTSD typically exhibit exaggerated suppression of cortisol in the stress response, suggesting a hypersensitivity to HPA negative feedback. In rats, exaggerated DEX suppression of corticosterone has also been demonstrated in the SPS model, but as with the clinical reports, this generalization was formed with evidence from predominately male populations. While our data replicate the expected effect in male rats, our data indicate that females exposed to SPS do not show the enhanced DEX-induced CORT suppression typical of SPS-exposed males in response to stress. This lack of an enhanced DEX suppression is akin to a "DEX non-suppression" phenotype typically associated with depression. Thus, enhanced DEX suppression of CORT (in males) and no DEX suppression of CORT (in females) may represent two core phenotypes of PTSD that may be segregated by gender. The acoustic startle response (ASR) measures the enhanced arousal associated with PTSD and, as expected, we found to be exaggerated in SPS-exposed males. However, we find that females normally become more reactive with repeated exposure to the startle stimulus, regardless of whether they have been exposed to SPS. These data indicate that female rats do not respond to SPS with the same phenotype established in male rats, indicating that the traumatic stress response manifests differently in male and female rats. Understanding the mechanisms underlying different types of PTSD response patterns is crucial in determining effective diagnostic measures and individual treatment.

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Poster

710. Mood Disorders Animal Models II

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Program#/Poster#: 710.25/Y28

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Reduced expression of RalBP1 decreases behavioral despair in mice

Authors: *S. H. YOON, Y.-S. BAE, K.-Y. PARK, M.-H. KIM;
Dept. of Physiol., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: RalBP1/RLIP76, a multifunctional protein that interacts with the small GTPase RalA and RalB, is widely expressed in the mammalian brain. Previous studies have shown that RalBP1 is associated with excitatory and inhibition synaptic functions in the central neurons. However, behavioral role of RalBP1 in the mammalian brain is unknown. In the present study, we investigated behavioral consequences of reduced expression of RalBP1 with mice hypomorphic for RalBP1 (RalBP1-/-, ~18% compared to WT mice). RalBP1-/- mice displayed decreased despair-like behaviors and were less immobile during the tail suspension test and the Porsolt forced swim test. Interestingly, however, anhedonia in the sucrose consumption test as well as anxiety levels in the elevated plus and zero maze tests were not changed in RalBP1-/- mice. In addition, reduced expression of RalBP1 had no effect on learning and memory, social interaction behaviors, and motor functions. These results suggest that RalBP1 mediated signaling regulates emotional states and that RalBP1 may be a new target for the treatment of depression.

Disclosures: S.H. Yoon: None. **Y. Bae:** None. **K. Park:** None. **M. Kim:** None.

Poster

710. Mood Disorders Animal Models II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Heep Fellowship in Neuroscience at Texas A&M University

Title: Melanocortin-5 receptor control of depressive behavior and brain mRNA expression in mice

Authors: *J. L. SHANNONHOUSE[1], A. V. MAYOROV[3], D. C. YORK[4], V. J. HRUBY[3], C. MORGAN[2];
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Abstract: Depression is a debilitating illness estimated to affect 6.6% of American adults every year. Melanocortin signaling disrupts rodent, and potentially human, emotional status, but roles for specific melanocortin receptors (MCR) have not been studied extensively. We investigated the role of the melanocortin-5 receptor (MC5R) in murine depressive behavior. MC5R knockout (KO) mice exhibited less depression-related immobility than did wild-type (WT) mice in the tail suspension test (TST), but no genotypic differences in motoric control behaviors were detected in home cages. TST immobility was elevated by intracerebroventricular infusion of selective and non-selective melanocortin agonists (FFM1-60 and NDP-MSH, respectively). Infusion of non-selective MCR antagonist, H2784, reduced immobility. Moreover, MC5R deficiency enhanced the efficacy of systemically injected antidepressant, imipramine. MC5R deficiency's effects on gene expression in the ventral striatum and dorsal hippocampus, two regions heavily implicated in depressive behavior, were measured. These results suggest that forebrain MC5R activation mediates depressive effects of stress.

Disclosures: J.L. Shannonhouse: None. **A.V. Mayorov:** None. **D.C. York:** None. **V.J. Hruby:** None. **C. Morgan:** None.

Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: R01MH086828

Title: Elevated corticosterone mediates the behavioral and neurobiological effects of chronic stress at rat hippocampal temporoammonic-CA1 synapses

Authors: *M. D. KVARTA[1], K. E. BRADBROOK[2], H. M. DANTRASSY[2], A. M. BAILEY[2], S. M. THOMPSON[1];

[1]Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Psychology, St. Mary's Col. of Maryland, St. Mary's City, MD

Abstract: Stress based models of depression, like Chronic Unpredictable Stress (CUS), cause a number of neurobiological changes at temporoammonic synapses onto hippocampal CA1 neurons (TA-CA1) and generate a depressive-like behavioral phenotype, reversible by antidepressant treatment. Responsible for spatial memory consolidation, the TA-CA1 synapse is also a useful archetype of stress- and antidepressant-sensitive excitatory synapses. We hypothesized that chronic stress-induced elevation of corticosterone (CORT) is necessary and sufficient to cause the synaptic correlates of CUS at stress labile synapses and that CORT-induced modulation of AMPAR expression and function cause depressive-like changes in behavior. In rats, we blunted CUS-induced elevations of CORT, verified by serum RIA and fecal pellet ELISA, with metyrapone (MET 50mg/kg i.p.) prior to each stressor. MET prevented loss of sucrose preference (SP), a measure of anhedonia. In a novelty suppressed feeding paradigm, MET+CUS rats displayed reduced latency compared to vehicle (VEH)+CUS. We observed that MET prevented decreases in the AMPAR component of field (f)EPSPs and GluA1 expression at TA-CA1 synapses. MET also attenuated the CUS-induced augmentation of potentiation of TA-CA1 responses by 5HT1BR agonists, compared to slices from VEH+CUS animals. CUS results in impaired spatial memory consolidation, much like a lesion of TA-CA1 synapses. Rats were trained in the Morris Water Maze, exposed to CUS during the consolidation period, and then retested after CUS. VEH+CUS rats demonstrated impaired consolidation compared to control, while MET+CUS rats did not, as measured by path length and latency to platform. MET thus prevented this cognitive deficit caused by CUS. We then determined whether elevated CORT is sufficient to recapitulate the effects of CUS by administering CORT via the rats' drinking water (50μg/mL) for 3 weeks. Chronic CORT, in the absence of stress, caused SP loss, decreased AMPAR mediated TA-CA1 fEPSPs and GluA1 expression, and increased irreversible fEPSP potentiation by 5HT1BR agonists, all consistent with the CUS phenotype. Inhibiting CUS-induced CORT elevations prevented, whereas exogenous CORT caused, the same pattern of behavioral and physiological changes observed after CUS. Taken together, we conclude that chronic CORT elevation is sufficient and necessary for the neurobiological changes of stress-induced depression at a model glutamatergic synapse. Manipulating this mediator of neurobiological changes provides insight into the pathogenesis of depressive neuropsychiatric disorders and potential therapeutic approaches.

Disclosures: **M.D. Kvarta:** None. **K.E. Bradbrook:** None. **H.M. Dantrassy:** None. **A.M. Bailey:** None. **S.M. Thompson:** None.

Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIDA Grant R01

China Scholarship Council

Title: Role of suprachiasmatic nucleus (SCN) neuronal firing in mood-related behaviors

Authors: *H. ZHANG[1,2], M. M. SIDOR[1], R. W. LOGAN[1], C. A. MCCLUNG[1];
[1]Dept. of Psychiatry, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Tsinghua Univ., Beijing, China

Abstract: Background: Studies have shown that altered circadian rhythms may play a role in mediating mood symptoms. The suprachiasmatic nucleus (SCN) is the pace maker of circadian rhythms. Here, we employ optogenetics to manipulate SCN neural activity to directly study the relationship between circadian rhythms and mood. Methods: VIP::ChR2 mice were used whereby the light-sensitive ion channel, channelrhodopsin (ChR2), is specifically expressed in vasoactive intestinal peptide (VIP) neurons of the SCN to enable direct control of SCN neurons with light. Mice were implanted with a fiber optic directly situated above the SCN to deliver light to ChR2-expressing VIP neurons. Then we will perform a series of behavioral tests, including the forced swim test and sucrose preference test to measure depression-like behavior and the open field, elevated plus maze, and light-dark box to measure anxiety-like behavior following optic-induced disruption of SCN neural activity and rhythms. Results: After determined that laser light delivered directly into the brain (independent of opsin expression) did not activate SCN neurons, as well as confirmed the co-localization of ChR2 with VIP and the ability of light to activate VIP neurons using immunohistochemistry, we were able to use SCN optical stimulation to change locomotor activity rhythms (which was considered as evidence of circadian rhythm change) in VIP::ChR2 mouse. Excitation of the SCN instantly increases locomotor activity and vice versa. Conclusion: These proof of principle experiments demonstrate that the SCN can be effectively targeted for optogenetic control of SCN neuronal activity. Further more, diurnal-specific perturbation of SCN neural activity can predictably shift locomotor activity rhythms.

Disclosures: H. Zhang: None. **M.M. Sidor:** None. **R.W. Logan:** None. **C.A. McClung:** None.

Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH R01 MH097714 01A1

Title: Role of the ventral tegmental area for sex-difference effects of social stress: Importance of topographical specificity, stressor predictability and interactions with nonapeptide systems

Authors: *G. GREENBERG[1], M. Q. STEINMAN[2], I. E. DOIG[2], S. P. YEH[2], B. C. TRAINOR[1];
[1]Neurosci. Grad. Group, [2]UC Davis Dept. of Psychology, Davis, CA

Abstract: The mesolimbic dopamine pathway is an important modulator of motivational components of social behavior. Interestingly, ventral tegmental area (VTA) projections to the nucleus accumbens have important effects for both rewarding aspects of social behavior as well as aversive contexts. One hypothesis is that subpopulations of neurons within the VTA respond to these contrasting stimuli. In addition to topographical specificity, dopaminergic neuron response can depend on the timing or predictability of a stimulus. Social defeat stress induces depressive-like symptoms linked to changes in VTA dopaminergic neuron excitability in male mice. Here, we examined effects of social defeat stress on neuronal activity within dorsal-ventral subdivisions of the VTA in male and female California mice. We used cFos and tyrosine hydroxylase (TH) double-label immunohistochemistry to indirectly measure dopaminergic neuron activity one hour following either a single or three episodes of social defeat stress. After a single episode of stress, there were no differences in the number of TH-positive cells or TH-fos colocalizations in the VTA. We examined autogrooming behavior immediately before each episode of defeat as index of stress reactivity. Autogrooming in the homecage increased prior to a third defeat episode compared prior to a first defeat episode, suggesting that mice anticipated the onset of the stressor. One hour after a third episode of defeat, stressed females had significantly more TH-cFos colocalizations and TH-positive neurons than control females in the ventral but not dorsal VTA. Dopaminergic and nonapeptide systems, specifically oxytocin (OT) and arginine-vasopressin (AVP), independently have great importance for responses to social stimuli. We found a significant, positive correlation between TH-fos positive neurons in the ventral VTA and OT-fos positive neurons in the paraventricular nucleus (PVN) of the hypothalamus following a third defeat episode. There was also a nonsignificant trend for a positive correlation between ventral VTA TH-fos positive neurons and PVN AVP-fos neurons. Oxytocin fibers were detectable in VTA but not AVP fibers. We used additional mice to examine

social interaction two weeks after three defeat episodes. Both OT and AVP can activate vasopressin V1a receptors (V1aR). We found stress significantly increased ligand-site availability for V1aR in the VTA of male and female mice following social interaction testing two weeks following defeat, suggesting a long-term effect of social stress on VTA signaling. Ongoing studies will determine if increased dopaminergic neuron activity in the ventral VTA is a long-lasting effect.

Disclosures: G. Greenberg: None. **M.Q. Steinman:** None. **I.E. Doig:** None. **S.P. Yeh:** None. **B.C. Trainor:** None.

Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: MH097714

MH095253

Title: Defeat stress induces sex-specific changes in neuropeptide receptor expression in the nucleus accumbens

Authors: N. C. DUQUE[1], M. Q. STEINMAN[2], R. HAO[1], K. L. CAMPI[1], G. D. GREENBERG[1], S. A. LAREDO[1], K. L. BALES[1], *B. C. TRAINOR[1];
[1]Univ. of California -Davis, DAVIS, CA; [2]Univ. of California -Davis, Davis, CA

Abstract: Oxytocin (OT) has important effects on social behaviors and coping responses to stress, and the discovery that intranasally administered oxytocin is behaviorally active in humans has sparked an intense interest in therapeutic use of oxytocin. However, there is growing evidence that intranasal OT has different behavioral effects in clinical populations compared to healthy controls. A common result is that effects of OT are weakened in individuals with childhood trauma. Studying the monogamous California mouse, we examined the effects of defeat stress on OT neurons in the hypothalamus as well as oxytocin receptor (OTR) and vasopressin V1a receptor (V1aR). During defeat stress, the number of OT/fos (but not vasopressin/fos) positive cells in the paraventricular nucleus was negatively correlated with the number of fos positive cells in the nucleus accumbens (NAc). Interestingly, two weeks after defeat stress there were significant decreases in OTR expression in the NAc of both males and females. In contrast, defeat increased the expression of V1aR receptor in NAc of females but not males. No changes in receptor expression were detected in lateral septum or amygdala. These changes may explain why intranasal OT reverses social withdrawal in stressed males but exacerbates social withdrawal in stressed females. Consistent with this hypothesis, OT mRNA is positively correlated with social interaction in females naïve to defeat but negatively correlated with social interaction in females exposed to defeat. Together, these data suggest that defeat induced neuroplasticity in neuropeptide receptor expression in the NAc may have important effects on how OT modulates social behavior.

Disclosures: **N.C. Duque:** None. **B.C. Trainor:** None. **M.Q. Steinman:** None. **R. Hao:** None. **K.L. Campi:** None. **G.D. Greenberg:** None. **S.A. Laredo:** None. **K.L. Bales:** None.

Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: R01MH086828

T32GM008181

T32NS007375

Title: Rapid antidepressant action of an alpha5 selective benzodiazepine partial inverse agonist: Restoration of excitatory synaptic strength

Authors: J. M. FISCHELL, T. A. LEGATES, M. D. KVARTA, A. M. VAN DYKE, *S. M. THOMPSON;
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Abstract: Depression is one of the top ten causes of mortality and morbidity worldwide, however its etiology remains unknown. Current therapeutic treatments based on monoamine reuptake inhibition are effective in only a subset of patients and, when effective, work slowly. There is increasing evidence, including data from our own lab, that a weakening of excitatory synaptic transmission between multiple brain regions is induced in chronic stress models of depression-related behaviors. Ketamine, an NMDAR antagonist, has recently gained widespread attention as a novel fast-acting antidepressant, but its utility is limited by psychotomimetic and dissociative side effects. Ketamine's antidepressant effect is thought to be mediated by a rapid increase in network activation and subsequent persistent activity-dependent strengthening of pathologically weakened synapses. We hypothesized that partial inverse agonists of the benzodiazepine binding site of α5-containing GABA-A receptors, which act as negative allosteric modulators of GABAR function, would also promote network activity and thereby strengthen excitatory synapses. Expression of the α5 subunit is restricted to extrasynaptic sites in cortical and hippocampal pyramidal cells, potentially minimizing undesirable side effects. Rats exposed to either chronic unpredictable stress or chronic restraint stress showed depression-like changes in behavior, including loss of sucrose preference and decreased social interaction. We observed that a single injection of one such compound, L-655,078, produced a restoration of

both depression related behaviors within 24 hrs in rats subjected previously to chronic stress, but had no effect in controls. Hippocampal slices taken from stressed rats also showed a decrease in the AMPAR-mediated field (f)EPSPs at the stress-sensitive temporoammonic-CA1 (TA-CA1) synapse, with a corresponding decrease in expression and phosphorylation of GluA1 at serine 831. 24 hours after injection of L-655,078, there was also restoration of the strength of AMPAR-mediated synaptic excitation and GluA1 expression and phosphorylation at TA-CA1 synapses. Injection of L-655,708 produced an immediate increase in oscillatory field potentials *in vivo* in area CA1 and in the nucleus accumbens in rats. Taken together, we conclude that L-655,078 exerts a rapid antidepressant action at the cellular, circuit, and behavioral levels, suggesting that this class of compounds may have clinical utility in the treatment of depression. The results support the hypothesis that defects in excitatory synapse contribute to the pathology of depression.

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Poster

711. Mood Disorders Animal Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 711.06/Z3

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH103296

Title: Early life exercise produces persistent neuroplastic changes within limbic regions involved in stress resistance

Authors: *A. MIKA[1], K. A. HULEN[1], C. A. BOUCHET[2], N. L. RUMIAN[1], A. K. HILLS[1], M. KEAG[1], D. BORCHERT[1], B. N. GREENWOOD[2], M. FLESHNER[1];
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Abstract: We have previously observed that exercise during early, sensitive developmental periods can alter the trajectory of brain development to produce long lasting resistance to the behavioral consequences of stress in adulthood. However, the neurobiological mechanisms by which this occurs are still unknown. A diverse body of literature demonstrates that neurotrophins such as brain derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF-2) not

only contribute to fundamental functions such as neuronal growth/development and survival but can also produce antidepressant and anxiolytic effects, contribute to the actions of antidepressant/anxiolytic drugs and modulate behavioral responses to stress. Furthermore, BDNF and FGF-2 are both upregulated following exercise and have been implicated as potential mediators of many of the adaptive behavioral and neuroplastic effects of exercise on the brain. It is therefore possible that these growth factors contribute to the persistent stress protective effects produced by early life exercise. The goals of the current study were to first, examine whether early life exercise can produce long-lasting alterations in BDNF and FGF-2 gene expression within limbic circuits regulating emotion/behavioral responses to stress, and second, whether brain regions displaying these patterns also demonstrate long lasting changes in neuronal structure and plasticity. Adult (PND 70) and juvenile (PND 24) male, Fisher (F344) rats were allowed access to a running wheel or remained sedentary for 6 weeks. Rats were sacrificed either immediately or 25 days after exercise cessation. Brains were removed and either processed for *in situ* hybridization for examination of gene expression, or in accordance with Golgi Stain protocols for examination of neuronal morphology. Preliminary analyses show that early life exercise produces persistent increases in BDNF mRNA expression within the hippocampus. Further quantification of brain regions involved in stress resistance, as well as quantification of FGF-2/changes in neuronal structure is currently underway. These data can provide insight into mechanisms by which exercise during critical developmental windows can alter brain development to produce a stress resistant phenotype.

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Poster

711. Mood Disorders Animal Models III

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Program#/Poster#: 711.07/Z4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Involvement of the CB1 cannabinoid receptor in the cognitive deficits associated with nicotine withdrawal

Authors: *R. SARAVIA[1], Á. FLORES[2], A. PLAZA-ZABALA[2], A. BUSQUETS-GARCÍA[2], A. PASTOR[3], R. DE LA TORRE[3], A. OZAITA[2], R. MALDONADO[2], F. BERRENDERO[2];

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Abstract: Abstinence from smoking produces a range of withdrawal symptoms including impaired attention and memory. Some studies suggest that relapse to tobacco use after a period of abstinence may occur to ameliorate these cognitive deficits. Moreover, poor cognitive performance during nicotine abstinence has been shown to predict more rapid smoking resumption. The objective of this study was to investigate the possible neurobiological mechanisms underlying this nicotine effect. Nicotine was administered by using Alzet osmotic minipumps (25 mg/kg/day) during 14 days in male C57BL/6 J mice. Withdrawal was precipitated by the administration of the nicotinic receptor antagonist, mecamylamine (2 mg/kg). A deficit in memory consolidation was observed by using the object recognition task when animals performed this test 24 hours after the precipitation of withdrawal, and this cognitive deficit was still present at least during 4 days. Interestingly, memory impairment was abolished by the administration of the cannabinoid receptor 1 (CB_1) antagonist rimonabant (1 mg/kg), and in CB_1 knockout mice. Moreover, chronic administration of rimonabant (1 mg/kg/day) during 4 days blocked the memory deficits 4 days after the precipitation of withdrawal. The metabotropic glutamate receptor 5 (mGluR5) is known to be involved in the biosynthesis of endocannabinoids. We observed that administration of the mGluR5 antagonist, MTEP (1 mg/kg), prevented the memory impairment associated with nicotine abstinence. An increase in the levels of 2-arachidonic, but not anandamide, was observed in whole brains after the precipitation of withdrawal. These results suggest that the activation of the CB_1 cannabinoid receptors could be, at least in part, responsible for the cognitive deficits observed during nicotine abstinence.

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Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NUI Maynooth John & Pat Hume Scholarship

Title: The effects of chronic fluoxetine administration on affective and neuroimmune changes associated with LPS-induced sepsis in mice

Authors: *S. T. ANDERSON, S. COMMINS, A. N. COOGAN;
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Abstract: Septic encephalopathy is a frequent complication of sepsis, involving neuroinflammation, compromised blood brain barrier stability, and altered neurotransmission. Upon recovery from the acute effects of sepsis, many patients show impaired cognitive functioning and high levels of depressive & anxious symptoms - accompanied by altered EEG activity and reduction in hippocampal volume - termed post-septic encephalopathy. There is relatively limited research into post-septic encephalopathy and no available treatment. We have previously shown that survivors of LPS-induced sepsis exhibit long lasting depressive- and anxiety-like behaviour, elevated microglial activation and reduced neural precursor cell proliferation. We therefore examined whether a chronic treatment with fluoxetine, a well known SSRI, could ameliorate affective and neuroimmune changes in post-septic animals. Approval for all experiments was granted by the NUI Maynooth Ethics Committee. In the present study adult male C57bl/6 mice received an i.p. injection of a 5mg/kg dose of LPS (serotype0111.B4, Escherichia Coli, Sigma Ireland,) or sterile saline (0.9%), after which they were allowed to recover for one week. Half of each group were then administered fluoxetine (10mg/kg) orally in drinking water for 28 days. After the 28th day of treatment, animals underwent behavioural testing in an object recognition task, tail suspension test, sucrose preference test and elevated plus maze. Results indicated that depressive- and anxiety-like behaviours present in post-septic animals not receiving fluoxetine were attenuated in mice administered the drug. As changes in neurogenesis appear important in mediating the effects of SSRIs, we administered BrdU (50mg/kg, i.p.) 24 hours before sampling to assess changes in NPC proliferation and also examined hippocampal levels of BDNF, a neurotrophin important for NPC survival and development which is down-regulated during depression and inflammation. Levels of microglial activation (IBA1) and immediate early gene expression (Egr-1) were also assessed within the hippocampus. These results illustrate the utility of fluoxetine in improving depressive-like behaviours post-sepsis, as well as the role of NPC proliferation, microglial activation and hippocampal BDNF levels in modulating these effects. Financial **Support:** NUI Maynooth John & Pat Hume Scholarship

Disclosures: S.T. Anderson: None. **S. Commins:** None. **A.N. Coogan:** None.

Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: A part of this study is the result of "Understanding of molecular and environmental bases for brain health" performed under the Strategic Research Program for Brain Sciences by MEXT.

Title: Glial dysfunction in the mouse habenula causes depressive behaviors and sleep disturbance

Authors: *W. CUI[1], H. MIZUKAMI[3], M. YANAGISAWA[1], T. AIDA[1], M. NOMURA[4], Y. ISOMURA[5], R. TAKAYANAGI[4], K. OZAWA[3], K. TANAKA[1,6,2], H. AIZAWA[1];
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Abstract: Lateral habenula (LHb) is an epithalamic nucleus regulating the monoaminergic neurons in the brain stem. Recently this nucleus has attracted a surge of interest in psychiatry because studies have reported the pathological activation of the habenula in patients with major depression and in animal models. Hyperactivation of LHb neurons plays a pivotal role in the pathophysiology of depression, but it remains unclear how habenular neurons are activated to cause various symptoms in depression. Taking the glutamatergic transmission onto LHb neurons into consideration, dysregulation of the extracellular glutamate concentration may be the reason why LHb neurons are hyperactivated. Astrocytes play a major role in the regulation of the excitatory neural transmission by controlling extracellular glutamate concentration. Therefore, we hypothesized that dysfunction of LHb astrocytes causes the behavioral deficits characteristic of depression. We examined the activity of neurons in habenular pathways and performed behavioral and sleep analyses in mice with pharmacological and genetic inhibition of the activity of the glutamate transporter GLT-1 expressed in the LHb astrocytes. The habenula-specific inhibition of GLT-1 increased the neuronal firing rate and the level of c-Fos expression in the LHb. Mice with reduced GLT-1 activity in the habenula exhibited a depressive phenotype in the tail suspension and novelty-suppressed feeding tests. These animals also displayed increased susceptibility to chronic social defeat stress, but with normal locomotor activity as shown in the open-field test. Intriguingly, the mice showed disinhibition of rapid eye movement sleep, which is a characteristic sleep pattern in patients with depression. In conclusion, our results provide the first evidence that disrupting glutamate clearance in habenular astrocytes increases neuronal excitability and depressive-like phenotypes in behaviors and sleep.

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Poster

711. Mood Disorders Animal Models III

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Title: Enhanced adult hippocampal neurogenesis via regulation of perforant path activity: A novel treatment for stress-induced depression?

Authors: *S. YUN[1], S. MUKHERJEE[1], C. E. KANG[2], B. L. ROTH[3], Y. HAN[2], D. M. CHETKOVICH[2], A. J. EISCH[1];
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Abstract: A major problem with current antidepressants is that ~50% of patients will relapse. A better understand of the neural circuitry underlying depressive behaviors and antidepressive treatments will help us develop more effective treatments for Major Depressive Disorder (MDD). Recent studies – including those showing decreased hippocampal dentate gyrus (DG) progenitors and neuron number in unmedicated humans with MDD, and the requirement of adult DG neurogenesis for certain effects of antidepressants in some strains of rodents – suggest that

enhancement of hippocampal neurogenesis may be a useful treatment for depression. Our hypothesis is that controlled enhancement of DG input via the glutamatergic perforant path (PP) is antidepressive via increased DG neurogenesis. While entorhinal cortex (Ent) deep brain stimulation and thus PP activation improves spatial memory and enhances DG neurogenesis (Stone et al., 2011), it is unknown whether Ent stimulation can also be antidepressive. We used two independent methods to stimulate PP activity and/or increase excitability of Ent Layer II stellate neurons in rodents: Ent knockdown of the brain-specific hyperpolarization-activated cyclic nucleotide-gated (HCN) channel auxiliary subunit TRIP8b, and PP stimulation via the chemogenetic Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology. Thus far, the results support our hypothesis. For example, knockdown of Ent TRIP8b increases hippocampal neurogenesis and promotes neuronal maturation, and also improves performance in behavioral measures of hippocampal dependent memory (e.g. CFC) and antidepressant efficacy (e.g. FST). We are currently examining whether enhanced stellate cell excitability – via stereotaxic infusion of AAV-hM3Dq-mcherry into Ent Layer II – enhances hippocampal neuroplasticity and is antidepressant. Also, given that the pathways regulating PP activity after stress and during depression are unknown, we are filling this major knowledge gap by determining the level of TRIP8b in select brain regions and recording fEPSPs in the molecular layer of the DG from rodents that underwent social defeat stress. These results will reveal whether highly promising targets for the treatment of depression – DG neurogenesis and PP activity – work together to regulate affective behaviors. In addition to exploring this novel connection and suggesting better paths for treatment, these aims will notably advance our understanding of the molecular, cellular, and neuro-circuit level regulation of depression-related behaviors.

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Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Development of the rat depression model related to selective white matter injury

Authors: ***H. ONO**[1], H. IMAI[1], S. MIYAWAKI[1], S. MIYATA[2], H. NAKATOMI[1], M. MIKUNI[2], M. FUKUDA[2], N. SAITO[1];
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Abstract: [Background] Depression is a significant contributor to the global burden of disease and recently, late onset depression (LOD) gets familiar with growing elderly population. The occurrence of white matter hyperintensities on T2-weighted magnetic resonance images is more frequent in patients with LOD, compared with early-onset depression. This fact indicates that white matter injuries (WMI) may provoke some stress vulnerability leading to depression. In this study, we have developed a selective WMI rat model with restraint stress (RS) to evaluate the correlation between the WMI and depression. [Method] Sprague-Dawley rats (302-380g, n=108) were used in this study. Selective WMI was induced under general anesthesia with bilateral endothelin-1 injection. Animals were randomly assigned to 4 groups: WMI with RS (group 1); sham operation with RS (group 2); WMI no RS (group 3); sham operation, no RS (group 4). Two weeks after surgery, group 1 and 2 animals received 2 hours of RS a day, for 13 days. Some animals in group 1 and 4 received escitalopram along the protocol. Body weight (BW) was recorded daily and blood samples were collected at three time points along the protocol. Animals underwent a forced swimming test (FST) on the day following the 13th RS day. Animals were euthanized after the FST, and brain sections analyzed. [Result] Conventional histopathology of the operated rat brain revealed the selective damage of the internal capsule. RS significantly suppressed weight gain in groups 1 and 2 compared with non RS groups. Moreover the change in BW over time in group 1 was significantly different from group 2. The body weight reduction in group 1 reversed with the administration of escitalopram. The corticosterone levels were elevated at the seventh stress day and returned to basal levels at the thirteenth day in group 1 and 2. The immobility time on the FST for group 1 was longer than that of other groups. [Discussion] Accompanied with WMI, repeated RS induced a reduction in weight gain and prolongation of immobility time in FST. These results provide preliminary evidence that WMI could influence stress vulnerability leading to depression. Additionally, selective serotonin reuptake inhibitor reversed the weight gain reduction. In order to use this model as depression rat model, further behavioral tests need to be added, but it is considered that this model represents some aspects of the depression related to the WMI, and may have a potential to contribute to the near future aging society.

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Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

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Title: PACAP transgenic mice in the three hit model of depression: The involvement of BNST - CRF, cpEW - Urocortin1 and DR - serotonin

Authors: *B. GASZNER[1], L. KOVÁCS[1], T. GASZNER[1], L. GÁSPÁR[1], D. REGLőDI[1,2], K. LőRINCZ[1], J. FARKAS[1], H. HASHIMOTO[3], V. KORMOS[4,5];
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Abstract: According to the three hit theory of depression genetic predisposition, epigenetic factors and stress precipitate the symptoms of major depression. In this work we aimed to set up and validate a mouse model for depression by the evaluation of behavioral abnormalities in forced swim test by the activity of the hypothalamus pituitary adrenal (HPA) axis. We also planned to study the contribution of corticotropin releasing factor (CRF) producing neurons in the bed nucleus of the stria terminalis (BNST) central amygdala. The possible role of the CRF-related urocortin1 (Ucn1) peptide containing neurons of the central projecting Edinger-Westphal nucleus (cpEW) and serotoninergic neurons in the dorsal raphe (DR) were also studied. For genetic predisposition we used of offspring of pairs of mice heterozygous for the gene of pituitary adenylate cyclase-activating polypeptide (PACAP). Litters were exposed to severe maternal separation to induce epigenetic changes vs. non-deprived or briefly separated controls. Half of adult offspring later was subjected to the chronic variable mild stress paradigm. We hypothesized that mice carrying all three risk factors will fail to adapt or show some maladaptive alterations supporting the validity of the model, and the CRF, Ucn1 and serotoninergic systems will be affected in their peptide content and/or neuronal activity. According to our results our stress paradigm was effective as in stressed groups the adrenal gland weights significantly increased, which rise was the greatest in mice with maternal separation history. Corticosterone measurements supported this, indicating the maladaptation of the HPA axis. Histological results revealed that maternally deprived mice exposed to chronic stress reacted with an increase in CRF

immunoreactive cell counts and specific signal density (SSD) in the oval BNST (BNSTov). In contrast, in the central nucleus of the amygdala, the chronic stress-induced increase in the CRF SSD was in maternally non-deprived mice observed only. Similarly, in maternally deprived mice we did not find increased neuronal activity by FosB in Ucn1 neurons and the stress induced increase in Ucn1 was abolished. Chronic stress decreased the serotonin SSD in the DR. Mice exposed to all risk factors showed increased immobility in forced swim test. The three hit theory of depression seems to be applicable in PACAP heterozygote mice, and it could be a promising model to study the pathophysiology of stress-related mood disorders. The elevated CRF contents in BNSTov neurons, and decreased Ucn1 neuronal activity moreover altered DR-serotonin suggests that multiple systems are affected in this model of mood disorders.

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Poster

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Support: NIH Grant MH101180

Title: Infralimbic prefrontal cortex modulation of dopaminergic system function in chronic mild stress model of depression

Authors: *J. L. MOREINES[1,2], Z. L. OWRUTSKY[1], A. A. GRACE[1];
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Abstract: Dopamine (DA) system dysfunction has received significant attention as a potential mediator of reward abnormalities in individuals with major depressive disorder (MDD). Converging evidence from multiple animal models of MDD suggest that rodents that exhibit depressive-related behaviors also exhibit a decrease in the population activity of dopamine neurons located in the ventral tegmental area (VTA) recorded *in vivo*. However, it is not known which afferent circuits may mediate the dopamine system down-regulation in MDD. The infralimbic prefrontal cortex (ILPFC) is the rodent homologue of human subgenual cingulate Brodmann Area 25, an area that has been established as critical to depression circuitry in

humans. Using *in vivo* electrophysiological recordings of identified VTA DA neurons, previous work from our lab has demonstrated that activation of the ILPFC leads to a reduction in the population activity of VTA DA neurons. In the present study, we assessed whether inactivation of the ILPFC could normalize DA system function in rodents that experienced 4-6 weeks of unpredictable chronic mild stress and exhibited reduced DA system population activity. We found that pharmacological inactivation of the ILPFC using tetrodotoxin microinjection resulted in disinhibition of the DA system, restoring VTA DA neuron population activity to levels seen in unstressed controls. Furthermore, this alteration restores normal behavioral function following stress-induced down-regulation of the DA system. Thus, in parallel to that found in human MDD patients, hyperactivity in the ILPFC of rodents appears to be a causative factor in the DA-related anhedonia observed in the chronic mild stress model of depression.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R01-MH068283

Title: Voluntary exercise prevents reductions of mTOR mRNA in the prefrontal cortex and hippocampus following exposure to uncontrollable stress

Authors: J. BURNS[1], C. A. BOUCHET[1], P. R. GHASEM[2], P. J. CLARK[2], J. J. HERRERA[2], E. A. SISNEROS[2], A. MIKA[2], M. FLESHNER[2], *B. N. GREENWOOD[1];
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Abstract: Previous research demonstrates benefits of exercise, including enhanced learning / memory and resistance against stress-related psychiatric disorders such as depression. Rodents allowed to run in wheels, for example, have improved hippocampus-dependent learning and memory, and are protected against the depression-like behavioral consequences of uncontrollable stress. The mechanisms underlying these beneficial effects of exercise, however, remain unknown. The mammalian target of rapamycin (mTOR) is a transcription-regulator important for cell growth, proliferation, and survival. mTOR has recently been implicated in enhancing

hippocampus-dependent memory and in providing antidepressant effects through actions in the prefrontal cortex (PFC). Given the emerging role of mTOR in memory-enhancing and antidepressant responses, it is possible that mTOR contributes to the beneficial effects of exercise. Despite this possibility, the effects of exercise on mTOR remain unknown. The present study sought to examine the effects of exercise and stress on mTOR in the hippocampus and PFC. Adult, male F344 rats either remained sedentary or were allowed voluntary access to running wheels for 6 weeks. After 6 weeks, half of each group was exposed to no stress or uncontrollable tail shock. Rats were sacrificed immediately following stress and levels of mTor mRNA were measured using *in situ* hybridization. Exercise by itself decreased levels of mTOR mRNA in the infralimbic and prelimbic regions of the PFC, but not the hippocampus. Stress reduced mTOR mRNA levels in all regions examined in sedentary rats, and this reduction was prevented by wheel running. In fact, stress increased levels of mTOR mRNA in the PFC of physically active rats. Analyses of mTOR protein levels with immunohistochemistry are currently underway. The elevated levels of mTOR mRNA following stressor exposure in physically active, compared to sedentary, rats could contribute to protection against the deleterious effects of stress on learning and memory and depression-like behavior. These data could provide insight into novel strategies for the treatment and prevention of stress-related disorders.

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Poster

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R01 MH086828

Title: Chronic fluoxetine treatment blocks synaptic potentiation of the Temporoammonic-CA1 synapses in the hippocampus

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Abstract: Elevation of serotonin (5-HT) levels with SSRIs is the major pharmacological treatment of depression. Nevertheless, SSRIs are effective in only a subset of patients and, when effective, work slowly and produce side effects. A better understanding of the effects of chronic SSRI administration is needed. Our laboratory has shown that 5-HT can potentiate certain excitatory synapses, including some that are weakened in depression models, and that this potentiation is necessary for the therapeutic action of antidepressants. In the hippocampus, specific activation of 5-HT_{1B} receptors selectively potentiates glutamatergic signaling in temporoammonic (TA) to CA1 pyramidal cell synapses in the stratum lacunosum-moleculare (SLM). This occurs due to phosphorylation of the AMPA receptor subunit GluA1 at serine 831 via recruitment of the phospholipase C/Ca^{2+}/Calmodulin-dependent Protein Kinase II (PLC/Ca^{2+}/CaMKII) signaling cascade. When rats are treated for 3 weeks with the SSRI fluoxetine, however, this 5-HT_{1B}R-mediated increase in synaptic transmission is lost. We hypothesized that the inability of a 5-HT_{1B} specific agonist to potentiate the TA-CA1 synapses in chronic fluoxetine treated animals is the result of chronic SSRI-induced elevation of 5-HT, resulting in maximally potentiation of synaptic strength. Consistent with this hypothesis, slices derived from chronic fluoxetine animals exhibited a deficit in TA-CA1 LTP following high frequency stimulation (4x100 pulses@100Hz; ±6% increase in field potential slope vs. 145% in control slices, $n = 6$, $p < 0.01$). Low frequency stimulation (900 pulses @3Hz) produced a mild synaptic depotentiation (±15% decrease in field potential slope, $n = 8$, $p < 0.05$) in slices taken from fluoxetine treated rats. However, high frequency stimulation failed to induce a repotentiation in these previously depotentiated slices (±13% increase in field potential slope $n = 8$, $p < 0.01$). Furthermore, we observed no difference in the strength of the AMPA receptor-mediated component of field (f)EPSPs at TA-CA1 synapses in slices taken from rats chronically treated with fluoxetine, compared to littermate controls. These results are thus inconsistent with our hypothesis. Western blotting is in progress to determine whether chronic fluoxetine causes changes in the expression of 5-HT_{1B}Rs, CaMKII, and GluA1 phosphorylation.

Disclosures: A.M. Van Dyke: None. **T.C. Francis:** None. **H. Chen:** None. **A.J. Kallarackal:** None. **X. Cai:** None. **S.M. Thompson:** None.

Poster

711. Mood Disorders Animal Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 711.16/Z13

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: T32NS007375

R01MH086828

Title: Direct versus indirect modulation of the nucleus accumbens by serotonin signaling

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Abstract: While selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressants, it remains unclear why and how elevating serotonin exerts antidepressant effects. The likelihood of depressive episodes is increased in individuals with more stressful life events; depressed patients report more stressful life events than non-depressed subjects. A common element linking stress with the therapeutic actions of antidepressants is their opposing effects on excitatory synapses. There is increasing evidence that chronic stress exerts deleterious effects on excitatory synapse structure and function in multiple brain regions that are associated with cognition and emotion, such as the hippocampus and nucleus accumbens (NAc). In the hippocampus, antidepressants exert an opposing action to promote excitatory synaptic transmission. Our lab has shown previously that chronic stress weakens excitatory synaptic transmission in the hippocampal temporoammonic -CA1 synapse, whereas acute and chronic administration of SSRIs acts to promote a 5HT1B receptor-dependent potentiation. What remains unclear is how serotonin signaling works to promote excitatory synaptic transmission in other brain regions involved in mood regulation. For instance, the hippocampus provides excitatory glutamatergic input to the NAc influencing its activity. Chronic stress inhibits excitation of medium spiny cells in the NAc as does brief activation of 5HT1B receptors. It is therefore unclear how modulation of serotonin levels with SSRIs acts on the NAc to relieve the symptoms of depression. Does the direct action of serotonin in the NAc promote reward signaling, or does serotonin act in an indirect manner by increasing input from the hippocampus to the NAc? We are using whole-cell electrophysiological recordings in the NAc shell to dissect the neural circuits that underlie potential serotonin mediated changes in excitatory synaptic transmission. Preliminary work has shown that modulation of serotonin signaling through application of the 5HT1B receptor preferring agonist anpirtoline increases spontaneous excitatory postsynaptic current frequency in the NAc shell in parasagittal slices in which hippocampal-NAc connections are partially intact. This appears to be regulated through a mechanism that is not intrinsic to the NAc because coronal slices containing only the NAc do not show this anpirtoline-induced change. We are currently dissecting the neural circuit and specific receptor subtype involved in this process and determining how this influences evoked responses.

Disclosures: T.A. LeGates: None. **S.M. Thompson:** None.

Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: R01-MH068283

Title: Exercise reward is independent of exercise controllability and involves the nigrostriatal dopamine pathway

Authors: *C. A. BOUCHET[1], J. J. HERRERA[2], P. R. GHASEM[2], S. M. ENGEL[2], T. WIEMAN[2], J. BURNS[1], P. CLARK[2], M. FLESHNER[2,3], B. N. GREENWOOD[1];
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Abstract: Dopamine (DA) reward circuits are implicated in stress-related disorders such as anxiety and depression. Exercise reduces the incidence of stress-related disorders, but the contribution of exercise reward to exercise-induced stress resistance is unknown. We have reported that the protective effects of exercise are independent of exercise controllability; whereby both voluntary and forced wheel running protect rats against behavioral consequences of stress. Voluntary exercise is a natural reward, but whether rats find forced wheel running rewarding is unknown. Moreover, the contribution of DA systems to exercise reward is not well characterized. The mesolimbic DA pathway is classically implicated in reward and is sensitive to voluntary exercise. Emerging data implicates the nigrostriatal DA pathway, traditionally associated with movement, in reward. The contribution of the nigrostriatal DA pathway to exercise reward has been difficult to determine due to its dual role in movement and reward. The goal of the current studies was to determine whether the rewarding effects of wheel running depend on its controllability and involve the nigrostriatal DA pathway. Male F344 rats (12 / grp) were divided into voluntary and forced groups. Rats in the forced group were placed in wheels that were rotated by motors on a predetermined schedule resembling the typical pattern of voluntary running. For 30 d, rats were moved into voluntary or forced wheels or, on alternating nights, an empty cage. Two hours after wheel or empty cage exposure, rats were placed on one distinct side of a conditioned place preference (CPP) chamber. One side was always paired with running (paired) and the opposite side was paired with the empty cage (unpaired). Results of probe tests conducted 10, 20 and 30 d after the start of CPP training indicated that both voluntary

and forced wheel running are rewarding. Rats spent more time on the side of the CPP chamber paired with exercise, regardless of controllability. After the final probe trial, and 24 hours after the last running exposure, rats were placed on either the paired or unpaired side and sacrificed 30 min later. Double fluorescent *in situ* hybridization (cfos / TH in midbrain regions and cfos / dynorphin in the dorsal and ventral striatum) revealed that re-exposure to the paired side elicited conditioned activation of both the mesolimbic and nigrostriatal DA pathways independent of differences in locomotor activity. These data suggest that voluntary and forced wheel running can be rewarding and these rewarding effects may involve the nigrostriatal DA pathway. The rewarding effects of exercise could contribute to exercise-induced stress resistance.

Disclosures: C.A. Bouchet: None. **J.J. Herrera:** None. **P.R. Ghasem:** None. **S.M. Engel:** None. **T. Wieman:** None. **J. Burns:** None. **P. Clark:** None. **M. Fleshner:** None. **B.N. Greenwood:** None.

Poster

711. Mood Disorders Animal Models III

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Support: MIUR (PRIN 2009 prot.2009BRMW4W_001)

Fondazione Cariplo Prog. 2011-0635

ECNP ECNP Research Grant for Young Scientist 2010

Title: Acute stress rapidly increases the readily releasable pool of glutamate vesicles in prefrontal and frontal cortex through non-genomic action of corticosterone

Authors: *M. POPOLI[1], L. MUSAZZI[1], G. TRECCANI[1], C. PEREGO[2], N. NAVA[3], M. MILANESE[5], T. BONIFACINO[5], J. LAMANNA[6], A. MALGAROLI[6], J. R. NYENGAARD[3], G. WEGENER[4], F. DRAGO[7], G. RACAGNI[1], G. BONANNO[5];
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Abstract: Stress and glucocorticoids alter glutamatergic transmission, and the outcome of stress may range from plasticity enhancing effects to noxious, maladaptive changes. We have previously demonstrated that acute stress rapidly increases glutamate release in prefrontal and frontal cortex via glucocorticoid receptor and accumulation of presynaptic SNARE complex (1). Here we compared the *ex vivo* effects of acute stress on glutamate release with those of *in vitro* application of corticosterone, to analyze whether acute effect of stress on glutamatergic transmission is mediated by local synaptic action of corticosterone. We found that acute stress increases both the readily releasable pool of vesicles and depolarization-evoked glutamate release, while application *in vitro* of corticosterone rapidly increases the RRP, an effect dependent on synaptic receptors for the hormone, but does not induce glutamate release for up to 20 min (2). These findings indicate that corticosterone mediates the enhancement of glutamate release induced by acute stress, and the rapid non-genomic action of the hormone is necessary but not sufficient for this effect. 1. Musazzi et al. (2010) PloS ONE 5(1):e8566. 2. Treccani G et al. (2014) Mol Psychiatry 19:433-43.

Disclosures: **M. Popoli:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Servier Pharma, Fidia. **L. Musazzi:** None. **G. Treccani:** None. **C. Perego:** None. **N. Nava:** None. **M. Milanese:** None. **T. Bonifacino:** None. **J. Lamanna:** None. **A. Malgaroli:** None. **J.R. Nyengaard:** None. **G. Wegener:** None. **F. Drago:** None. **G. Racagni:** None. **G. Bonanno:** None.

Poster

711. Mood Disorders Animal Models III

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Program#/Poster#: 711.19/Z16

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Sex differences in compulsive and affective functions in a non-induced compulsive-like mouse model

Authors: *S. MITRA[1], C. P. BASTOS[3], A. BULT-ITO[2];
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Abstract: Obsessive-compulsive disorder is a psychiatric disorder characterized by persistent obsessive thoughts and compulsive repetitive behaviors. A previous study showed that our inbred strains with compulsive nest-building behavior have face and predictive validity as a non-induced animal model for OCD-like behavior. Here we tested the hypothesis that there are sex differences in compulsive-like behavior, affective functions and in HPA axis response to restrained stress. To test this hypothesis we measured compulsive like behavior (Nest Building and marble burying) and affective functions like anxiety (Open field and Elevated plus maze) and depression (Forced swim test) in male and female mice of two BIG, SMALL and control strains. To determine HPA axis response, plasma corticosterone levels were measured in males and females of three different strains through ELISA. Results showed that there are significant sex differences between two BIG compulsive-like strains in nest building behavior ($p<0.001$), while marble burying showed no significant differences. Sex differences were observed in one of the BIG compulsive-like strain in elevated plus maze test ($p<0.001$) for anxiety and forced swim test for depression ($p<0.05$). Sex differences were also noticed for SMALL and control strains in elevated plus maze and forced swim tests ($p<0.05$). Plasma corticosterone levels differed between male and female BIG ($p<0.05$) and SMALL ($p<0.05$) strains. The results direct towards sex dependent variations in compulsive behavior, affective function and HPA axis response during OCD-like condition.

Disclosures: S. Mitra: None. **A. Bult-Ito:** None. **C.P. Bastos:** None.

Poster

711. Mood Disorders Animal Models III

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Mead Johnson Nutrition

Title: Dietary prebiotics increase Bifidobacterium spp. and Lactobacillus spp. in the gut and promote stress resistance

Authors: *N. L. RUMIAN[1], A. MIKA[1], B. N. GREENWOOD[2], H. E. DAY[3], D. BORCHERT[1], M. M. PATON[1], M. CHICHLOWSKI[4], B. M. BERG[4], M. FLESHNER[1];
[1]Univ. of Colorado Boulder, Boulder, CO; [2]Univ. of Colorado Denver, Denver, CO; [3]Ctr. for Neurosci., Boulder, CO; [4]Mead Johnson Pediatric Nutr. Inst., Evansville, IN

Abstract: Prebiotics, a form of non-digestible dietary fiber, can selectively promote the expansion of beneficial microbial species in the mammalian gut. Two common prebiotics, Galactooligosaccharides (GOS) and Polydextrose (PDX), can increase Bifidobacterium spp. and Lactobacillus spp., implicated in attenuating depressive and anxiety-like behavior and generating adaptive changes in brain serotonin circuits implicated in mood. We therefore tested whether dietary GOS and PDX initiated during the juvenile period, would attenuate anxiety and depressive-like behavior produced by stressor exposure and produce changes in brain serotonin receptors. Juvenile Fisher rats (PND 24, n=9/grp) were fed a diet containing GOS/PDX (7.0 g/kg each) for either 4 or 9 weeks. Fecal samples were collected after 4 weeks on the diet and were plated on Lactobacillus spp. and Bifidobacterium spp. specific media. Rats were then either exposed to inescapable tail shock stress (IS; 100 1.5mA tail shocks; a stressor that reliably produces anxiety-like behavior) or remained undisturbed in their home cages. Subsequently, rats were either sacrificed immediately, or twenty-four h later, tested for anxiety/depressive-like behavior using shock-elicited freezing and shuttle-box escape tests. Fecal cultures confirmed that 4 weeks of GOS/PDX increased Lactobacillus spp. and Bifidobacterium spp. in fecal samples and preliminary data demonstrated increased mRNA expression of 5-HT1a receptors in the dorsal raphe nucleus (DRN), a brain region involved in regulating anxiety. Quantification of additional targets is currently underway. Both 4 and 9 weeks of diet containing GOS/PDX protected against the behavioral consequences of IS. These results show that GOS/PDX promote stress resistance in Juvenile Fisher rats, and suggest that gut bacteria may modulate serotonergic circuits regulating mood. Supported by Mead Johnson Nutrition.

Disclosures: N.L. Rumian: None. **A. Mika:** None. **B.N. Greenwood:** None. **H.E. Day:** None. **D. Borchert:** None. **M.M. Paton:** None. **M. Chichlowski:** None. **B.M. Berg:** None. **M. Fleshner:** None.

Poster

711. Mood Disorders Animal Models III

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Call for PhD projects 2011 DCN

Hersenstichting kleine subsidie 2013(1)-24

Title: Developmental delay in serotonin transporter deficient rats

Authors: *Y. KROEZE[1], S. JANSSEN[1], B. DIRVEN[1], H. ZHOU[2], J. HOMBERG[1];
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Abstract: Central nervous system serotonergic circuits are thought to play an important role in the pathophysiology of mood disorders, especially depression. One major risk factor for depression is a regulatory variation in the serotonin transporter (5-HTT) gene. The short (s) allelic variant of the serotonin transporter-linked polymorphic region (5-HTTLPR) is associated with reduced 5-HTT protein availability, anxiety-related traits and increased risk for depression in adulthood. However, insights in the effects of the 5-HTTLPR s-variant on early-life behavior and gene expression are limited. 5-HTT is transiently expressed in corticolimbic and somatosensory cortical non-serotonergic neurons during embryonic and early postnatal life. This transient 5-HTT expression is thought to maintain stable serotonin levels during critical developmental phases. Given the importance of 5-HTT during development, we hypothesize that 5-HTT deficiency has negative consequences on early-life behavior and can also affect expression of neurodevelopmental genes. To test this hypothesis, we used the 5-HTT knockout rat to assess their development of reflexes and behavior and gene expression pattern. This 5-HTT knockout rat lacks a functional 5-HTT and thereby models s-allele carriers. It has been shown that anxiety and depression-like phenotypes displayed by adult 5-HTT knockout rats more or less resemble the behavior in s-allele carriers. Up to now, we have assessed behavior during early development and found that 5-HTT knockout rats show a delay in developing reflexes, like negative geotaxis, vibrissa placement and righting reflex. Furthermore, these rats show impaired motor coordination, muscle strength, olfactory discrimination and grooming behavior. We are now performing the RNA-seq experiments at early-life (first 3 postnatal weeks), adolescence (postnatal day (PND) 35) and adulthood (PND70) in 5-HTT knockout and wildtype rats. In conclusion, reduced 5-HTT function can cause a delay in early life development, which may predispose to the adult anxiety- and depression-related phenotypes. We anticipate that early-life intervention in s-allele carriers has the potency to reduce risk for these adult phenotypes.

Disclosures: Y. Kroeze: None. **S. Janssen:** None. **B. Dirven:** None. **H. Zhou:** None. **J. Homberg:** None.

Poster

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FCT - PEst-C/SAU/LA0001/2013-2014

QREN - CENTRO-07-ST24-FEDER-002006

NARSAD

CNPq - Ciência sem Fronteiras

Title: The genetic deletion of adenosine A2A receptors selectively from amygdala neurons prevents mood-related modifications in rats subjected to repeated restraint stress

Authors: *N. GONCALVES[1], N. M. MACHADO[1], C. M. SOUZA[1], A. P. SIMÕES[1], R. A. CUNHA[1,2];
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Abstract: Mood disorders are the main cause of disability worldwide. The main contributing factor is the chronic exposure to stressful situations, leading to a constellation of behavioral modifications that involve alterations of amygdalar circuits. Since stress-induced mood dysfunctions are prevented by selective adenosine A2AR antagonists (e.g. SCH58621), and repeated stress upregulates A2AR in amygdalar nerve terminals, we now probed if A2AR silencing only in the amygdala is sufficient to control behavioral and functional modifications caused by repeated restraint stress. Male adult rats were injected bilaterally in the amygdala with lentiviral vectors encoding either a short hairpin for A2AR silencing (shA2AR) or a control (shCTR). One week after, rats were handled (control) or subjected to 2 weeks of repeated restraint stress (4 h daily). All rats (n=9-13) were evaluated in biochemical parameters (weight gain, plasma corticosterone [CORT]), in anxiety-like (elevated plus maze [EPM]) and depressive-like (forced swim test [FST]) behavioral paradigms, and functionally in amygdala

circuitries (long-term potentiation [LTP]). Restrained rats injected with shCTR displayed, compared to controls, a reduction in weight gain (39±7g versus 67±5g, $p<0.001$), a 6-fold increase in CORT levels (925±342ng/mL versus 162±43ng/mL), an increase of anxiety-like behavior (6.0±1.9% open arm exploration time versus 14.2±4.8%), decreased climbing/swimming time ratio in the FST (2.1±0.3 versus 3.3±0.4), and increased amygdalar synaptic plasticity, namely LTP (241.5±30.6% versus 167.3±20.9% in controls, n=3). Silencing A2AR in the amygdala did not affect body weight alterations, but attenuated the increase of CORT levels, prevented the anxiogenic (16.0±5.3%) and depressive-like (3.1±0.3) behaviors, and reduced amygdalar LTP (176.8±33.2%, n=3) in restrained rats. These results show that ablating amygdalar A2AR is sufficient to prevent stress-induced behavioral and functional modifications associated with depressive-like conditions; this prompts targeting amygdalar A2AR as a promising strategy to manage mood-related disorders.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Mead Johnson Nutrition

Title: Multi-nutrient experimental diet increases stress-protective gut bacteria, improves early life NREM sleep architecture, and enhances REM sleep rebound following an acute stressor

Authors: ***R. S. THOMPSON**[1], A. MIKA[1], R. ROLLER[1], B. N. GREENWOOD[1], M. CHICHLOWSKI[2], B. M. BERG[2], M. FLESHNER[1];
[1]Univ. Colorado Boul, BOULDER, CO; [2]Mead Johnson Pediatric Nutr. Inst., Evansville, IN

Abstract: Stressor exposure can produce anxiety-like and depressive-like behaviors, and disruptions in the sleep/wake cycle. Prebiotics, a form of non-digestible dietary fiber, can selectively promote the expansion of specific microbial species (i.e., Bifidobacterium spp. and Lactobacillus spp.) in the mammalian gut that promote stress resistance and prevent stress-evoked anxiety and depression. It may be possible, therefore, that a diet containing prebiotics can prevent stress-evoked anxiety and depression and protect the sleep/wake cycle. Thus we tested

the hypothesis that a diet containing prebiotics and other nutrients would increase Bifidobacterium spp. and Lactobacillus spp. species, modulate the sleep/wake cycle prior to stress exposure and provide protective effects to the sleep/wake cycle following stress exposure. Male F344 rats, postnatal day 24 (P24), were placed on either prebiotic (Galactooligosaccharides, Polydextrose, Lactoferrin, Docosahexaenoic acid, Arachidonic acid, and Milk Fat Globule Membrane 10) or non-prebiotic diet ad-libitum. In the first experiment (n = 32), body weights and food consumption were measured and weekly fecal samples were collected. In the second experiment (n = 32), biotelemetry devices were implanted on P59, to examine real-time differences in the sleep/wake cycle across rodent development due to prebiotic diet. Rats were exposed to an acute inescapable stressor on P87 in order to examine the potential protective effects of prebiotic diet on stress-induced disruptions of the sleep/wake cycle. Fecal cultures confirmed that rats fed the prebiotic diet had increases in stress-protective Bifidobacteria and Lactobacillus when compared to rats fed the non-prebiotic diet. In the second experiment, rats fed the prebiotic diet had greater NREM sleep consolidation in early adulthood (P71, P72) compared with the non-prebiotic diet. In addition, rats fed the prebiotic diet also displayed enhanced REM rebound following acute stress exposure (P87) compared to rats fed the non-prebiotic diet. These results demonstrate that administration of a diet containing prebiotics increased stress-protective gut bacteria, increased NREM sleep consolidation, and conferred stress-protective effects on REM sleep following an acute stressor. Our results suggest that modulation of the gut commensal flora with prebiotics improves sleep architecture and may help reduce the incidence of disruptions to the sleep/wake cycle induced by stress exposure.
Supported by Mead Johnson Nutrition.

Disclosures: R.S. Thompson: None. **A. Mika:** None. **R. Roller:** None. **B.N. Greenwood:** None. **M. Chichlowski:** None. **B.M. Berg:** None. **M. Fleshner:** None.

Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMHD Grant P20MD002290

Title: Chronic mild stress induces depression-like symptoms in high anxiety female rats: Differential fos and 5HT1A expression

Authors: *R. LOTT, M. SWEENEY, B. MASON, R. MEJIA, S. T. DONALDSON;
Univ. of Massachusetts Boston, Boston, MA

Abstract: Depression and anxiety disorders have considerable overlap in clinical settings, suggesting common underpinnings that can be investigated in reliable animal models. In the current study, we used 40 outbred female Long-Evans rats (PND145-150) bred for high (HAn) and low (LAn) anxiety-like behavior (ALB) to determine if they exhibited depression-like symptoms (DLS). Animals were tested initially on the elevated plus maze (EPM; percent open arm (%OA) time) and forced swim test (FST; immobility, floating) to measure ALB and DLS. After an initial FST, we exposed animals to an unpredictable three-week chronic mild stress (CMS) protocol that included reversed light cycle, 24-h food deprivation, tilted home cage and soiled bedding unpredictable exposures. During CMS, we assessed weight, piloerection, and sweet food consumption at the end of each of the three weeks; we also did vaginal lavages immediately after CMS and again four weeks later to see if estrous cycles had been disrupted. Finally, the animals were given a final FST, transcardially perfused and the brains were processed for free-floating immunohistochemistry (IHC) to measure Fos and 5HT1A expression in the hypothalamus and hippocampus. Rats phenotyped as HAn showed greater ALB (i.e., lower %OA time and entries) on the EPM, but active coping styles in the FST (i.e., more mobility and swimming). After CMS, the HAn group showed even more mobility and swimming, less weight gain and fewer Fruit Loops™ consumed. Fur analysis indicated more piloerection in treatment groups, on the face and back, across the CMS treatment weeks. Evaluation of estrous cycles revealed that HAn animals, in general, showed greater consecutive cycling (both immediately after CMS and 1 month later) compared to LAn females. Initial IHC results show increased Fos positive cells in the hypothalamus of LAn animals following CMS but a *decrease* in HAn females. 5HT1A positive cells were higher in the hypothalamus and hippocampus for HAn control females, though in the CMS groups, expression increased to the same levels for both anxiety profiles; there were no differences noted in the hippocampus. In summary, our results indicate that the outbred HAn phenotypes show robust ALB, exhibit active coping styles in the FST, and yet are more sensitive to CMS-induced weight loss and decline in palatable food ingestion. Interestingly, the HAn profile 'protected' animals from the CMS stalling of the estrous cycle and the elevated neural response to the FST. However, HAn animals do show higher basal levels of hippocampal and hypothalamic 5HTIA expression, which may contribute to group differences in DLS following the CMS protocol.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: FONDECYT 1140108

Title: Serum microvesicles as potential biomarkers for psychiatric diseases

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Abstract: Mayor depressive disorder (MDD) is a multifactorial disease with increasing evidence for the existence of sub-types. The diagnosis of subtypes with objective criteria is an actual challenge and would help with the election of appropriate treatments. Animal models of MDD are based on exposure to chronic stress. Previous work in our laboratory demonstrated that two MDD animal models based on movement reduction, either by restriction (RS) in small cages or immobilization (IS) in plastic bags, were able to induce depressive-like behaviors that were reverted differentially by two families of antidepressant drugs, fluoxetine (a specific serotonin reuptake inhibitor) or reboxetine (a specific noradrenaline reuptake inhibitor). We therefore searched for protein markers in the cerebrospinal fluid (CSF) and blood serum to help in the characterization of both models. In the CSF, the metabolic enzyme fructose 1, 6-bisphosphate aldolase C (AldoC), expressed in forebrain astrocytes and in cerebellar Purkinje cells, was highly increased after RS, but not after IS. We then isolated microvesicles from the serum of control, RS and RI animals. This fraction contained AldoC. Moreover, recombinant AldoC, expressed exclusively in forebrain astrocytes, could be collected in plasma microvesicles. Our results show that serum microvesicles of cerebrocortical origin may reflect many aspects of CNS function and might provide a novel and useful tool to identify protein or miRNA markers in psychiatric diseases.

Disclosures: C. Gomez-Molina: None. **E. Ampuero:** None. **A. Luarte:** None. **U. Wyneken:** None.

Poster

711. Mood Disorders Animal Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 711.26/Z23

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH

The Ellison Medical Foundation New Scholar in Aging and a Whitehall Foundation grant

Title: Klf9 is a novel transcriptional regulator of resilience to chronic stress

Authors: A. BESNARD[1,4], *T. LANGBERG[1,4], S. LEVINSON[1,4], K. M. SCOBIE[5], E. D. LEONARDO[6], R. HEN[6], A. SAHAY[2,3,4,7];
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Abstract: Major depressive disorder (MDD) affects multiple neural circuits subserving anhedonia, encoding, and stress-coping behaviors and has greater prevalence in women. Although MDD arises from an interaction of genes that moderate vulnerability and environmental factors such as stress, how these factors converge upon neural circuitry to alter behavior is poorly understood. Identification of molecular mechanisms that moderate the effects of stress on neural circuits to influence behavior may catalyze the generation of novel antidepressants. Here, we identify Kruppel-like factor 9 (Klf9) as a novel regulator of synaptic connectivity that moderates the effects of chronic stress on depression-like behaviors. Previous work showed Klf9 expression to be upregulated by glucocorticoids and in the hippocampus of patients with MDD. Using novel transgenic tools by which Klf9 expression can be inducibly and reversibly silenced in the forebrain, we investigated the effects of Klf9 down-regulation on depression-like behaviors in a mouse model of chronic restraint stress (CRS). Silencing Klf9 in adulthood did not affect contextual encoding or depression-like behaviors at baseline, but produced antidepressant-like behavioral responses in the sucrose preference and forced-swim tests and prevented stress-induced enhancement of fear memory following CRS. Interestingly, these effects were only seen in females and not males, mirroring Klf9 silencing dependent-reversal of CRS induced changes in dendritic spines in ventral CA1. Furthermore, Klf-9 downregulation blunted the corticosterone response to an acute stressor challenge in CRS female mice. Together, these observations suggest that Klf9-silencing promotes resilience to chronic stress and targeting Klf9 may harbor therapeutic potential for MDD.

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Poster

711. Mood Disorders Animal Models III

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Program#/Poster#: 711.27/Z24

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Mercer University College of Pharmacy

Title: Validation of adolescent chronic restraint stress as a model of depression in adult female Sprague Dawley rats

Authors: M. HIBICKE, *R. L. HAYSLETT;
Mercer Univ. Coll Pharma, ATLANTA, GA

Abstract: Chronic restraint stress has been validated as a model of depression in adult rats, but depressive-like behaviors tend to normalize after restraint sessions end. However, chronic stress during times of rapid development, such as neonatal stress, has been shown to result in behavioral and physiological differences in adulthood, and can be used to model psychiatric disorders. Childhood and adolescent stress is a risk factor for developing adult depression, which disproportionately afflicts women from pubescence to menopause. In this pilot study we sought to validate adolescent chronic restraint stress as an animal model for depression in adulthood and measured behavioral changes as a result of chronic restraint. Early adolescent female Sprague Dawley rats, 32-40 days old (n=6), were restrained for one hour daily at unpredictable times during the light cycle for 12 consecutive days. The rats were placed in well-ventilated restraining tubes small enough to prevent them from moving freely, but not small enough to create physical discomfort. Restraint sessions occurred in individual holding cages in a brightly lit room. The length of restraint sessions and duration of restraint period were chosen based on previous reports. Control animals (n=6) were handled briefly and placed back their home cages while their counterparts were exposed to restraint stress. Weight gain was monitored through the duration of the study. Chronic restraint elicited a stress response as evidenced by a significant reduction in weight gain of the restrained animals during the 12-day restraint period compared to controls. This reduction in weight gain normalized to control levels after the restraint period ended. In addition, the effect of adolescent chronic restraint on anhedonic-like behavior in adulthood was assessed. Fifty-three to sixty-two day-old rats that had been exposed to chronic restraint drank significantly less volumes of a 2% sucrose solution compared to controls as measured by the sucrose preference test. These preliminary results suggest that chronic restraint during adolescence precipitated depressive-like behavior in adult female rats and may be a putative animal model of depression.

Disclosures: M. Hibicke: None. **R.L. Hayslett:** None.

Poster

711. Mood Disorders Animal Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 711.28/Z25

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Aging an corticosterone administration differently alter mouse emotionality and gene expression in the dentate gyrus

Authors: *J.-P. GUILLOUX[1], D. FELICE[1], Y. LI[2], A. L. PEHRSON[2], I. MENDEZ-DAVID[1], A. M. GARDIER[1], C. SANCHEZ[2], D. J. P. DAVID[1];
[1]Univ. Paris Sud, EA3544, Faculté De Pharmacie, Chatenay-Malabry, France; [2]Lundbeck Res. USA, Paramus, NJ

Abstract: Aging is commonly associated with increased incidence of depression and anxiety disorders and declines in cognitive functions. Furthermore, stress is commonly associated with development of depression and anxiety. Here we investigated the role of age and chronic corticosterone administration on depression/anxiety-related behavior in mice in neurogenesis dependent/independent tasks. Molecular changes associated with age and/or corticosterone administration were analyzed using microarray. Groups of 2- and 16-months old mice exposed to low chronic corticosterone administration (4 weeks) in the drinking water and untreated controls were evaluated in tests of an anxiety- depression- related phenotype, i.e.: open field, elevated plus maze, novelty suppressed feeding (NSF), saccharin preference. Gene expression changes in the dentate gyrus were analyzed using Affymetrix MouseGene Arrays. Age did not affect depression/anxiety-related behaviors, while chronic corticosterone treatment induced a depression/anxiety phenotype with a greater effect in young animals, increasing latency to feed in NSF and decreasing saccharin preference. Aging effects on gene expression in the dentate gyrus slightly differed between untreated and corticosterone-treated animals, as 72% of the genes observed down or up-regulated in either condition showed similar directionality between groups. Comparatively, effects of chronic corticosterone treatment strongly differed between young and old animals. A chronic corticosterone treatment induced changes in expression of 386 and 475 genes specifically in young- and aged-animals respectively ($|\text{Fold Change}|>1.2$, $p<0.05$), with very few genes (28) commonly altered in both conditions. Gene expression related to adult hippocampal neurogenesis were affected by both aging and chronic corticosterone administration. Interestingly, we also observed that age related-genes were more susceptible to cort-induced changes. Moreover, we showed that CORT related-genes in young animals were

also more susceptible to age-induced changes. Taken together, chronic corticosterone treatment induced a depression/anxiety-related phenotype at both ages, although to a lesser extends in aged mice. These results were associated with molecular changes in the dentate gyrus confirming the overlap between the CORT-related pathology and the effects of aging on gene expression.

Disclosures: **J. Guilloux:** 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.; Lundbeck Research USA. **D. Felice:** 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.; Lundbeck Research USA. **D.J.P. David:** 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.; Lundbeck Research USA. **A.M. Gardier:** 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.; Lundbeck Research USA. **I. Mendez-David:** 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.; Lundbeck Research USA. **C. Sanchez:** A. Employment/Salary (full or part-time):; Lundbeck Research, USA. **A.L. Pehrson:** A. Employment/Salary (full or part-time):; Lundbeck Research, USA. **Y. Li:** A. Employment/Salary (full or part-time):; Lundbeck Research, USA.

Poster

712. Alcohol: Developmental Effects

Location: Halls A-C

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Program#/Poster#: 712.01/Z26

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIGMS COBRE Grant 1P20GM103653 - 01A1 to AYK

Title: Immediate and long-term impact of developmental alcohol exposure on BDNF and TrkB in the frontal cortex

Authors: K. J. CRISS[1], V. S. PALAMARCHOUK[2], K. E. BOSCHEN[2], *A. Y. KLINTSOVA[2]; [1]Psychology, [2]Univ. of Delaware, NEWARK, DE

Abstract: Deficits in executive functioning are considered a “hallmark feature” of Fetal Alcohol Spectrum Disorders (FASD; Green et al., 2009) amongst the wide range of neuroanatomical, cognitive, behavioral, and physical deficits that can result from exposure to alcohol *in utero*. Thus, damage to the frontal cortex (FC) is strongly implicated. FC is especially vulnerable to insult by alcohol during the “brain growth spurt” (third trimester equivalent in humans and first two postnatal weeks in rodents). Previous work in our lab demonstrated that developmental alcohol exposure altered PFC neuroplasticity, including spine morphology and dendritic complexity of basilar dendrites of Layer III neurons when examined on PD26-30 (Hamilton et al., 2010). This study investigates the immediate and long-term effects of binge-like alcohol exposure during early development on levels of brain-derived neurotrophic factor (BDNF) protein, its receptor, TrkB tyrosine kinase (TrkB), and total protein in FC. On postnatal days (PD) 4-9, alcohol-exposed rat pups (AE) were intragastrically intubated twice daily with ethanol, receiving 5.25 g/kg/day. Sham-intubated (SI) animals were intubated alongside AE pups without the infusion of solution, while suckle control (SC) animals were left undisturbed. For short-term analysis, animals were sacrificed on PD10. Animals used for the long-term study were assigned to one of three housing conditions from PD30-72: social housing (SH), wheel running only (WRWR), or twelve days of WR followed by exposure to a complex environment (WREC). Cortical tissue and trunk blood were harvested on PD10 and 72. A sandwich Enzyme-Linked Immunosorbant Assay (ELISA) was performed to quantitate BDNF and TrkB in FC at both time points. The Pierce Coomassie Protein Assay was used for quantification of total protein in extracts from the brain tissue. Preliminary findings suggests that on PD10, BDNF was significantly elevated in AE animals, while TrkB was decreased in SI pups, namely in females. A trend for increased total protein in AE pups was also evident. A positive correlation between plasma/brain corticosterone level and BDNF and an inverse correlation between corticosterone level and TrkB protein were found in the FC at PD10 and PD72; cortical BDNF and TrkB were not impacted by treatment or housing. Together, these data provide novel insight into the short and long-term effects of alcohol exposure and stress due to the intubation method of alcohol administration on the development of frontal cortex.

Disclosures: K.J. Criss: None. **A.Y. Klintsova:** None. **V.S. Palamarchouk:** None. **K.E. Boschen:** None.

Poster

712. Alcohol: Developmental Effects

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Program#/Poster#: 712.02/Z27

Topic: C.17. Drugs of Abuse and Addiction

Support: 5P20GM103653-02

Title: Moderate *in utero* alcohol exposure results in sex-dependent inflammation in the developing rat brain

Authors: *R. DIMATTEO, J. M. SCHWARZ;
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Abstract: Activation of the developing immune system can have significant consequences for neural development and long-term neural function. Recent evidence indicates that alcohol exposure can activate the peripheral immune system and microglia, the immune cells in the brain. The purpose of these experiments was to determine whether moderate alcohol exposure early in pregnancy would cause sex-dependent inflammation in the fetal brain with long-term consequences. Pregnant Sprague-Dawley rats were assigned to the following treatment groups: no gavage, gavage with water, or gavage with 50% ethanol twice a day from embryonic days 10 to 16, the equivalent of the human first trimester. Analysis of blood alcohol concentrations produced a consistent 0.08% blood alcohol concentration for approximately 8 hours each day. On E17, the hippocampus and cortex of both male and female rat pups was collected for the analysis of pro-inflammatory cytokines and chemokines using real-time PCR. Alcohol exposure increased the expression of cytokines in the tumor necrosis factor superfamily, including TNF-alpha, TNF-SF13, and TNF-SF13B in the hippocampus-cortex of females but not males. Two chemokines, CCL2 and CCL20, revealed opposing expression trends in males and females such that levels decreased in males and increased in females following alcohol treatment. We have also collected the placenta, maternal brain, maternal liver, and spleen for on-going analysis, because maternal or placental inflammation caused by moderate alcohol may also influence fetal brain development. In an on-going experiment, we repeated our paradigm and raised pups to adulthood in order to examine long-term changes in immune function and/or behavior. We found no effect of moderate alcohol exposure on pup weights, average litter size, or postpartum maternal behavior. One cohort of treated rats was tested for baseline differences in anxiety or cognition. We found no significant effects of sex or treatment group for the time spent in open arms of an elevated plus maze. In an open field test, females spent more time in the center of the open field; however alcohol-treated males explored the center significantly more than their water-treated counterparts. In a novel object recognition task, both control and alcohol treated offspring of both sexes were able to identify the novel object in this task. We predict that pups exposed to moderate prenatal alcohol may exhibit a stronger neuroimmune or immune response to LPS treatment in adulthood, which may precipitate cognitive deficits in these rats. These on-going studies will contribute to our understanding of the impact of alcohol on the developing brain and immune system.

Disclosures: **R. Dimatteo:** None. **J.M. Schwarz:** None.

Poster

712. Alcohol: Developmental Effects

Location: Halls A-C

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Program#/Poster#: 712.03/Z28

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant R37 AA08757

Title: The effects of pre-pregnancy maternal alcohol exposure in female rats on hypothalamic-pituitary-adrenal axis function in offspring

Authors: *L. G. CHASTAIN, S. JABBAR, M. A. CABRERA, D. K. SARKAR;
The Endocrine Program, Dept. of Animal Sci., Rutgers, The State Univ. of New Jersey, New Brunswick, NJ

Abstract: Developmental exposure to alcohol results in significant disruption of hypothalamic-pituitary-adrenal (HPA) axis functioning both in humans and in animal models. Previous studies have shown fetal exposure to alcohol results in endocrine and behavioral disruptions in the exposed rat which persist into adulthood. While it is known that alcohol exposure during pregnancy results in HPA axis disruption in the exposed fetus, it is not known if alcohol exposure in adult females prior to pregnancy has any physiological effects on the HPA axis of the female's offspring. The following studies sought to determine if chronic exposure to alcohol in adult females prior to pregnancy alters HPA axis function in adult offspring. Adult female CD Fischer rats were assigned to one of three treatment groups: 1) the control group (n = 8) receiving rat chow and water ad libitum (AD), 2) the alcohol fed group (AF, n = 12) receiving a liquid diet containing ethanol (7.8%), or 3) the pair fed (PF, n = 12) group receiving an isocaloric liquid control diet. After 30 days of treatment, all animals were returned to the ad libitum diet for about 10 days then bred with untreated male CD Fischer rats. Adult F1 progeny (4-6 months of age) of AD, AF, and PF females were tested for changes in HPA axis function by measuring anxiety-like behaviors in the elevated plus maze (EPM) and open field (OF) test, endocrine response to physical restraint stress, and adrenal gland weight. Male progeny of AF females (AFM) and male progeny of PF females (PFM) showed significantly more time spent in the closed arms of the EPM compared to male progeny of AD females (ADM). In the OF test, AFM rats showed a reduced latency to escape to the wall when placed in the center of the OF compared to ADM and PFM groups, indicating increased anxiety-like behavior in male progeny of alcohol-exposed females. Baseline plasma concentrations of corticosterone (CORT) did not differ between ADM, PFM, and AFM groups, but PFM and AFM groups showed reduced levels of CORT during physical restraint stress, suggesting a blunted endocrine response to stress in

these groups. Plasma CORT concentrations did not differ among groups at timepoints following restraint stress. Finally, AFM rats showed increased adrenal gland weight compared to PFM and ADM rats. Together, these studies suggest chronic maternal exposure to alcohol before pregnancy results in altered HPA axis function in adult male offspring.

Disclosures: L.G. Chastain: None. **S. Jabbar:** None. **M.A. Cabrera:** None. **D.K. Sarkar:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA006916

NIH Grant AA007568

Title: Effects of developmental ethanol exposures in wildtype and p53-null mice on transcriptional and epigenetic regulation of DNA damage, DNA repair, cell cycle, and cell death processes

Authors: *F. A. MIDDLETON[1], M. CAMARGO[1], C. IGNACIO[1], S. HICKS[1], S. MOONEY[2]; [1]SUNY Upstate Med. Univ., SYRACUSE, NY; [2]Pediatrics, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Developmental alcohol exposure induces DNA damage in the CNS, and affected neurons may upregulate DNA repair mechanisms, alter their cell cycle and cell fate, or undergo apoptosis as a result. p53 plays a major role in the regulation of these processes. Here, we characterize changes in these p53-regulated processes at the DNA, RNA and protein levels following ethanol exposure using *in vivo* and *in vitro* models in the presence and absence of p53. First, wildtype (WT) and p53 knockout (KO) mice were injected with ethanol on P7 and euthanized 8 hours later. Confirmation of changes in apoptosis was performed using immunohistochemical analyses. Then, we examined alterations in mRNA and microRNA expression, DNA methylation, and expression and binding of DNA repair proteins. mRNA was quantified using a custom RNA-Seq panel designed to interrogate 275 genes related to DNA repair, cell cycle and cell fate, or apoptosis. The RNA used in these studies was purified from a

total of 192 laser-dissected samples of somatosensory cortex (including 4 different lamina) and hippocampus (including the CA1 and dentate gyrus). Interestingly, most of the significant expression changes were distinct for specific brain regions, and only a few showed generalized trends across all the areas. However, most genes in the dentate gyrus showed strongly opposing effects of ethanol in the WT and p53 KO mice, suggesting possible compensatory mechanisms in the absence of p53. Within the somatosensory cortex, ethanol-induced changes were most common in layer 2/3. mRNA analysis of mouse primary cultures exposed to 400 mg/dL ethanol for 24 and 48 hours confirmed the changes in many of the same genes. Analysis of microRNA profiles from the same cultures also strongly supported the involvement of p53-related processes. Finally, genome-wide DNA analysis using methylation profiling and chromatin immunoprecipitation for specific DNA repair proteins (ChIP-Seq) identified several genes that may be a focus of ethanol epigenetic and regulatory actions. Together, these data strongly support the involvement of critical p53-dependent processes and targets in regulating the developmental effects of ethanol on the brain.

Disclosures: F.A. Middleton: None. **M. Camargo:** None. **C. Ignacio:** None. **S. Hicks:** None. **S. Mooney:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: Korea government (MSIP) 2011-0030049

Title: A comparative transcriptomic study of mouse embryonic neural stem cell differentiation under ethanol treatment

Authors: C. MANDAL, J. PARK, M. CHOI, S. KIM, K. PARK, J. CHAI, Y. LEE, K. JUNG, *Y.-G. CHAI;
Dept. of Mol. & Life Sci., Hanyang Univ., Ansan, Korea, Republic of

Abstract: Neural stem cells (NSCs) can be differentiated into one of three cell lineages: neurons, astrocytes or, oligodendrocytes. Ethanol has previously been reported to alter the cell fate of NSCs. To explore the molecular mechanism underlying this phenomenon, we performed a comparative transcriptomic analysis of mouse NSC differentiation in the presence or absence of

ethanol. In this study, NSCs from the forebrains of embryonic day 15 mouse embryos were differentiated for two days in the presence or absence of 50 mM ethanol. Microarray analysis showed that ethanol altered the expression of 496 genes, 56 of which were up-regulated; 440 were down-regulated. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed the association of the following altered genes in the Wnt signaling pathway: Wnt5a, Csnk2a1, Tcf7l2, Ccnd2, Tbl1xr1 and Rac2. Quantitative real-time PCR analysis also demonstrated the relative expression levels of these genes. Wnt signaling plays an important role in fetal brain development. Therefore, ethanol-induced alterations may contribute to improper development of the brain. Our data could be a significant resource for elucidating the mechanism of action of ethanol in the developing brain.

Disclosures: C. Mandal: None. **J. Park:** None. **M. Choi:** None. **S. Kim:** None. **K. Park:** None. **J. Chai:** None. **Y. Lee:** None. **K. Jung:** None. **Y. Chai:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: Chenery Research Grant

Title: Effects of a single intoxication event on striatal medium spiny neuron morphology during the early postnatal period in mice

Authors: T. WESTON[1], C. LOWERS[1], A. BAMFO[1], J. SOKOLOWSKI[2], *E. B. CLABOUGH[1];
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Abstract: Exposure to ethanol in early development can result in physiological alterations to neuronal pathways, and can specifically affect brain regions related to reward processing. The striatum appears to be particularly vulnerable to this neuromodulation, as ethanol can depress both dopaminergic and GABAergic transmission. Long-term early ethanol exposure can cause dose-dependent cell death and induce morphological changes in neurons. In addition, a single intoxication event is enough to generate widespread neurodegeneration in many brain regions. We investigated the effect of a single ethanol intoxication episode on striatal medium spiny

neuron (MSN) morphology during brain development. Animals were exposed to brief, high levels of ethanol during the early postnatal period of brain development. Mice were administered 2 doses of EtOH (2.5 g/kg) on postnatal day 7 (P7) two hours apart. After 24 hours, brains were removed and processed for Golgi-Cox staining. MSNs from the caudate/putamen were analyzed for changes in dendritic complexity and soma size. These data characterize immediate alterations in the morphology of MSNs as a consequence of neonatal exposure to a transient dose of EtOH. The results enhance our understanding about the impact of EtOH exposure on surviving neurons, and provide clues to explain the increased self-administration of drugs of abuse seen in animals exposed to ethanol during brain development.

Disclosures: T. Weston: None. **A. Bamfo:** None. **C. Lowers:** None. **E.B. Clabough:** None. **J. Sokolowski:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH COBRE Grant P30GM103400

NIAAA Grant AA019462

Title: Resting state functional connectivity of male and female long-evans rats is altered as a result of moderate prenatal ethanol exposure

Authors: *C. I. RODRIGUEZ[1], S. DAVIES[2], V. CALHOUN[4,3], D. SAVAGE[2], D. HAMILTON[1];
[1]Psychology, [2]Neurosciences, [3]Electrical and Computer Engin., The Univ. of New Mexico, Albuquerque, NM; [4]The Mind Res. Network, Albuquerque, NM

Abstract: Heavy ethanol exposure during prenatal brain development leads to profound morphological, neurological, and behavioral consequences. Moderate prenatal ethanol exposure (MPEE) leads to less profound and subtler effects. MPEE research has largely targeted single brain regions or local networks. In this study, we examined whole-brain functional connectivity using group independent component analysis (GICA) applied to functional MRI data. Male and female Long-Evans rats were exposed to 5% ethanol or saccharin throughout gestation. BOLD

signals were acquired during a 10 minute echoplanar imaging sequence performed with anesthetized (1.0-2.3% isoflurane) adult rats. After image preprocessing, spatial independent component analysis (ICA) was performed using the Infomax algorithm implemented in the GIFT toolbox. A total of 24 non-artifactual components were retained for analysis of connectivity by component cross-correlations. Components were observed in frontal cortex, hippocampus, striatum, thalamus, cerebellum and several posterior cortical regions. Positive correlations within same-brain regions were observed for both diet conditions, however, saccharin rats had more significant correlations within striatal, hippocampal, and cerebellar components. Seperate two-way ANOVAs on component cross-correlations indicated several interactions between prenatal treatment and sex factors, most of which were found in cortico-hippocampal and midbrain-hippocampal relationships. An overall reduction (26%) in connectivity of MPEE rats was also observed. MPEE primarily affected cortico-hippocampal, cortico-thalamic, and cortico-cerebellar relationships. Further analyses in male rats indicate reductions in connectivity in cortico-hippocampal and cortico-thalamic relationships in the MPEE condition. The majority of significant differences in female rats were observed in cortico-hippocampal relationships. However, many of these differences suggest an increase, rather than a decrease, in cortico-hippocampal connectivity MPEE females. A two-way ANOVA of binned and averaged spectral power revealed a number of statistically significant interactions and main effects of sex and prenatal treatment condition. Additional analyses revealed alterations in low frequency spectral power for a small set of components across treatment and sex conditions. Our results indicate that MPEE alters resting state functional connectivity of networks involved in motor, sensory, and cognitive functions.

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Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NICHD R01HD069238

Title: Exposure to alcohol and alterations in the miRNA network in the human fetal brain in early and mid-gestation

Authors: *N. DARBINIAN, K. I. MUHAMMAD, N. MERABOVA, G. TATEVOSIAN, M. SELZER, L. GOETZL;
Shriners Hosp. Pediatric Res. Ctr., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Introduction: We hypothesized that miRNAs play an important role in brain development and neurological diseases secondary to alcohol exposure. miRNA changes have been described in animal models of fetal alcohol syndrome (FAS) but not in human samples with *in vivo* EtOH exposure.
Methods: We quantified expression levels of 90 miRNAs in human fetal brain samples and paired maternal blood collected at the time of elective pregnancy termination. Samples with and without significant EtOH exposure were compared using miRNA microarray techniques and verified by quantitative RT-PCR. Our selected microarray panel targets miRNAs associated with neurodevelopmental disorders including: autism, schizophrenia, anxiety disorders and Tourette's Syndrome, all disorders with increased incidence in children with FAS.
Results: Changes in miRNAs with gestational age (GA) were observed in control subjects between the first and second trimester, likely representing normal development. In contrast, EtOH exposure induced dysregulation of miRNA signaling in both the fetal brain and maternal blood. In first trimester samples, screened miRNAs were significantly up-regulated (2.5-5.5 fold) in the fetal brain and down-regulated in maternal blood (3.5 - 6.5 fold). In second trimester samples, EtOH exposure was associated with smaller effects in the opposite direction: inhibition in fetal brain specimens and slight up regulation in maternal blood. There was a positive correlation between paired maternal and fetal specimens for the number of miRNAs at early GA, and less or negative correlation at late GA.
Conclusions: Patterns of fetal brain miRNA expression across GA are complex and require further study. Brains of fetuses exposed to EtOH show abnormal expression of miRNAs whose dysregulation has been associated with several neurodevelopment disorders. This suggests a possible mechanism by which FAS leads to increased risk for these same neurodevelopment disorders.

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Poster

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Title: Cytokine and chemokine expression and microglial activation induced by ethanol in a mouse model of FASD are prevented by the PPAR-gamma agonist pioglitazone

Authors: *C. J. KANE, J. W. JOHNSON, J. C. DOUGLAS, K. D. PHELAN, P. D. DREW; Dept Neurobio. & Developmental Sci., Univ. Arkansas Med. Sci., LITTLE ROCK, AR

Abstract: Fetal alcohol spectrum disorders (FASD) result from maternal alcohol consumption during pregnancy and are the leading cause of mental retardation in the U.S. There is currently no effective treatment that targets the causes of these disorders. Using the neonatal mouse model of FASD, characterized by significant neuronal loss and long term behavioral deficits, we previously demonstrated that microglia are highly vulnerable targets of ethanol pathogenesis in the developing brain. Specifically, ethanol exposure causes loss of microglial cells and the surviving microglia express morphological changes suggestive of microglial activation and neuroinflammation. In addition, we demonstrated that neuroprotective and anti-inflammatory pharmaceutical PPAR-γ agonists block ethanol pathogenesis in both neurons and microglia. The present study analyzed cytokine and chemokine expression and microglial morphology in this model to determine the consequences of ethanol-induced microglial activation in the developing brain. Further, we determined whether treatment with the PPAR-γ agonist pioglitazone would prevent ethanol-induced pathogenic changes. Mice were treated with ethanol or vehicle by gavage on postnatal days (PD) 4-9. Animals also received either water or pioglitazone by gavage on PD4-9. On PD10, the hippocampus, cerebellum, and cerebral cortex were isolated. Analysis of gene expression revealed that ethanol induced expression of IL-1β, TNF-α, and CCL2 (MCP-1) mRNA in the hippocampus and the cerebellum. Ethanol induced expression of IL-1β and TNF-α in the cerebral cortex. Treatment with pioglitazone prevented the ethanol-induced increase in cytokine and chemokine expression to the level of that in control animals. Ethanol induced a morphological change in microglia in these brain regions indicative of activation, which was blocked by pioglitazone. These findings shed light on an important new potential mechanism underlying the neuropathology of FASD. They indicate that ethanol activates microglia to a pro-inflammatory stage and also increases the expression of neuroinflammatory molecules in diverse regions of the developing brain. Further, the anti-inflammatory and neuroprotective PPAR-γ agonist pioglitazone blocked these effects. It is proposed that microglial activation and inflammatory molecules expressed as a result of ethanol treatment during brain development contribute to the sequelae associated with FASD. Thus, the FDA approved pharmaceutical agent pioglitazone and anti-inflammatory pharmaceuticals more broadly have

potential as novel therapeutics for FASD. (Supported by NIH grants AA018834, AA18839 and AA19108)

Disclosures: C.J. Kane: None. **J.W. Johnson:** None. **J.C. Douglas:** None. **K.D. Phelan:** None. **P.D. Drew:** None.

Poster

712. Alcohol: Developmental Effects

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Program#/Poster#: 712.10/Z35

Topic: C.17. Drugs of Abuse and Addiction

Support: ABMRF, The Foundation for Alcohol Research, grant 60034780

Title: NMDA receptor downstream signaling in the dorsal hippocampus of trace fear conditioned adult rats exposed to ethanol in early postnatal life

Authors: *M. J. GOODFELLOW[1], J. M. POCHIRO[2], D. H. LINDQUIST[1,2];
[1]Psychology, [2]Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Fetal alcohol spectrum disorder (FASD), which can result from gestational exposure to ethanol, is characterized by a variety of physical and cognitive abnormalities (Mattson et al. 2011), including diminished learning and memory. Utilizing a rat model of binge-like, 3rd trimester-equivalent drinking, pups are administered ethanol (5 g/kg/day; 5E) across postnatal days (PD) 4-9. We have previously demonstrated impaired auditory trace fear conditioning (TFC) which requires association of a tone conditioned stimulus (CS) with a non-overlapping footshock unconditioned stimulus (US) in young adult (~PD70) 5E rats (Dupont et al. 2014). Acquisition of TFC depends on NMDA receptor (NMDAR)-dependent plasticity in the dorsal hippocampus (DH) (Misane et al., 2005), a brain region sensitive to the neurotoxic effects of postnatal ethanol (Livy et al., 2003). Additional work from our lab has established ethanol-induced changes in the ratio of NMDAR subunits in the DH of 5E rats, with NR2B reduced relative to NR2A, which could limit the induction of long-term potentiation (LTP) (Shouval et al., 2002). Dupont et al. (2014) also reported significant decreases in phosphorylated ERK1/2-positive neurons in the DH of 5E rats 1 h after TFC. ERK1/2 is activated as a consequence of NMDAR-gated Ca^{2+} influx and plays a key role in long-term memory consolidation (Peng et al. 2010). The current study was designed to assess the relationship between impaired TFC in 5E rats and putative alterations in NMDAR downstream signaling. In ongoing research, rats are

trained in TFC then submitted to further behavioral testing or sacrificed. Two proteins activated downstream of the NMDAR, important for LTP induction and maintenance, are quantified in DH via immunofluorescence at two post-TFC time points:10 or 120 min. At 10 min we are quantifying expression levels of the NMDAR NR2B subunit and phosphorylated α-Ca^{2+}/calmodulin-dependent protein kinase II (pαCaMKII). The interaction between synaptic NR2B and CaMKII activates a number of downstream signaling molecules, including ERK1/2 and GluR1 (Lisman, Yasuda & Raghavachari, 2012). At 120 min, two AMPA receptor (AMPAR) subunits are being quantified: GluR2, as a measure of total AMPAR expression, and phosphorylated GluR1 (pGluR1), which potentiates AMPAR kinetics. We hypothesize that pαCaMKII and/or pGluR1 will be reduced in 5E rats, linked, perhaps, to previously described alterations in NMDAR subunit composition, diminishing LTP induction and/or maintenance and contributing to observed TFC deficits.

Disclosures: M.J. Goodfellow: None. **J.M. Pochiro:** None. **D.H. Lindquist:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA AA015614

NIGMS 5K12GM088021

Title: The actions of alcohol on electrophysiological properties of gabaergic and putative serotonergic neurons in the dorsal raphe of developing mice

Authors: *R. A. MORTON[1], Y. YANAGAWA[2], F. C. VALENZUELA[1];
[1]Neurosciences, Univ. of New Mexico, Albuquerque, NM; [2]Dept. of Genet. and Behavioral Neurosci., Gunma Univ. Grad. Sch. of Med., Maebashi, Japan

Abstract: Serotonin (5-hydroxytryptamine (5-HT)) is a key modulatory neurotransmitter that is primarily expressed by neurons located within the raphe nucleus of the brain stem. Serotonergic neurons located within the dorsal raphe nucleus project throughout the forebrain, and receive GABAergic inhibition from local interneurons. The brain growth spurt and synaptogenesis that occurs during the human third trimester of pregnancy is equivalent to the first two weeks of post-

natal life in rodents. During this period, the neuronal circuits in the raphe nucleus undergo significant refinement. The goal of these studies is to characterize the electrophysiological properties of both local GABAergic and putative serotonergic neurons within the dorsal raphe during the third trimester-equivalent period (post-natal days (P) P5 - P16) and to test the sensitivity to acute ethanol exposure. GABAergic neurons were identified by the expression of the venus fluorescent protein (developed by Dr. Atsushi Miyawaki at RIKEN, Wako, Japan) driven by the VGAT promoter (Wang et al. 2009), and putative serotonergic neurons were identified by their anatomical location and the lack of venus expression. During this period, the cell capacitance of both GFP+ cells (61.56 ± 26.43 pF; n = 4) and GFP- cells (66.08 ± 23.78 pF; n = 5) remained relatively stable. Similarly, the membrane resistance of GFP+ cells (680.1 ± 285 MΩ; n = 4) and GFP- cells (640.8 ± 248 MΩ; n = 5) was similar and did not change during this developmental period. Loose cell-attached recordings indicated that 100% of GFP+ cells fire spontaneously at approximately 5.5 ± 3.8 Hz (n = 8), whereas only 33% of GFP- cells fire spontaneously and do so at 2.59 ± 1.34 Hz (n = 8). Current-clamp experiments suggest that GFP+ cells have a lower action potential threshold of ~55 mV (n = 4) versus ~47 mV (n = 4) for GFP- cells. Furthermore, a current injection of 100 pA resulted in a firing frequency of 11.3 Hz for GFP+ cell and 15.95 Hz for GFP- cells at P5 - P7. However, at P10 - P11 the firing frequency of GFP+ cells was 46.12 Hz and GFP- cells fired at 14.91 Hz. The basal frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) in both GFP + (1.5 Hz and 79.87 pA; n = 5) and GFP - (0.98 Hz and 107.25 pA; n = 5) cells were similar. In the presence of 50 mM ethanol the sIPSC frequency in the GFP - cells nearly doubled (194% ± 40; n = 5), whereas the sIPSCs are relatively un-affected in the GFP + cells (84.5 % ± 20; n = 4). These data suggest that ethanol preferentially alters the inhibitory transmission in putative serotonergic neurons during this critical developmental period.

Disclosures: **R.A. Morton:** None. **F.C. Valenzuela:** None. **Y. Yanagawa:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: A.10. Adolescent Development

Support: NIH Grant RO1AA013098

NIH Grant P50AA017823

Title: Possible contributions of GABAA and Kappa Opioid Receptor in transgenerational effects of prenatal ethanol exposure

Authors: *D. O. POPOOLA[1], M. E. NIZHNIKOV[2], N. E. SPEAR[2], N. M. CAMERON[2]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]Psychology, State Univ. of New York (SUNY), Binghamton, NY

Abstract: For decades, clinical and experimental research has provided evidence that prenatal ethanol exposure (PEE) modulates postnatal response to ethanol but the underlying mechanism remains unclear. Our laboratory recently reported transgenerationally transmitted behavioral effects of PEE. We found increased juvenile voluntary ethanol consumption in both prenatally treated (F1) and their ethanol-naïve offspring (F2). Also, in ethanol exposed F1 and F2 male adolescents, sensitivity to ethanol's sedative-hypnotic effects was attenuated. The γ-aminobutyric acid type-A (GABAA) and Kappa opioid (KO) systems are strongly implicated in ethanol-induced sedation-hypnosis and voluntary consumption. This study investigated PEE-induced alterations in these systems as potential mediators of PEE-induced behavioral alterations in prenatally treated F1 generation and ethanol-naïve F2 generation. To produce the F1 generation, ethanol (1g/kg) or water was administered to Sprague Dawley rats on gestational days 17-20 via gavage (i.g) while a control group remained undisturbed. Same-treatment adults from different litters were mated to produce an F2 generation that remained undisturbed through gestation. At postnatal day 42, male offspring's brains from both generations were collected. Cerebral cortex, cerebellum, amygdala and ventral tegmental area were micro-dissected for protein analysis, in whole cell and synaptosomal (P2) fraction, of GABAA α1 and α4 and kappa opioid receptor (KOR) using western blotting. In the cerebral cortex, F1 ethanol and water subjects expressed higher GABAA α1 in P2 but not whole cell analysis. Water but not ethanol subjects maintained this higher α1 expression in F2 generation compared to controls. KOR expression in the cerebral cortex was also significantly higher in ethanol and water groups compared to control, both in F1 and F2 generation P2 analysis. No difference was observed in whole cell analysis. There was no treatment effect on GABAA α4 expression in any region or fraction assessed, neither was there any effect of treatment in the other regions. These results indicate that PEE alters the GABAA and KO systems and such effects can persist across generations. Thus, these systems may be, at least in part, responsible for the behavioral effects. The data also suggest long-lasting effects of the i.g procedure possibly through handling-induced stress. As these results don't completely parallel the observed behavioral patterns, on-going investigations focus on elucidating their exact implications and other contributing factors. Neurosteroid activity and hypothalamic-pituitary-adrenal axis regulation are of prime focus.

Disclosures: D.O. Popoola: None. **M.E. Nizhnikov:** None. **N.E. Spear:** None. **N.M. Cameron:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 1P20GM103653 - 01A1

Title: Immediate and long-term impact of developmental alcohol exposure on BDNF gene expression and protein in the hippocampus

Authors: *K. E. BOSCHEN, V. PALAMARCHOUK, K. J. CRISS, T. L. ROTH, A. Y. KLINTSOVA;
Psychology, Univ. of Delaware, Newark, DE

Abstract: Prenatal alcohol exposure in humans results in a wide array of neuroanatomical, cognitive, behavioral, and physiological deficits. The hippocampus is particularly vulnerable to teratogenic insult during the “brain growth spurt” (third trimester equivalent in humans and first two postnatal weeks in rodents), potentially contributing to memory and cognitive deficits in humans following *in utero* alcohol exposure. Alcohol exposure during the third trimester equivalent has been shown to negatively impact hippocampal neuroplasticity, including dendritic morphology, LTP and adult neurogenesis. The current study investigates the effects of a binge-like alcohol exposure during early postnatal development on levels of brain-derived neurotrophic factor (BDNF), its receptor, TrkB tyrosine kinase (TrkB), and expression of exon-specific BDNF mRNA transcripts in infancy and adulthood. On postnatal days (PD) 4-9, alcohol-exposed rat pups (AE) were intragastrically intubated 2x daily with 5.25 g/kg/day ethanol. Sham-intubated (SI) animals were intubated alongside AE pups without liquid and suckle control (SC) animals were undisturbed. For short-term analysis, animals were sacrificed on PD10. Animals used for the long-term study were assigned to one of three housing conditions from PD30-72: social housing (SH), wheel running only (WRWR), or twelve days of WR followed by exposure to a complex environment (WREC) and sacrificed on PD72. The levels of BDNF and TrkB protein in the HPC were detected by sandwich ELISA. The Pierce Coomassie Protein Assay is used for quantification of total protein for prepared extracts from the brain tissue. Preliminary data suggests that on PD10, levels of BDNF and TrkB were significantly higher in the HPC of AE rats compare to SI controls. No significant effect of neonatal treatments was found at PD72 in SH animals. Gene expression of BDNF exons I, IV and IX (total) were analyzed using qPCR. Preliminary data suggests no alcohol-related changes to BDNF mRNA expression in the HPC at PD72 in SH rats and a significant increase in BDNF gene expression following WRWR but not

WREC. These data indicate that neonatal alcohol exposure has pronounced effects on basal BDNF and TrkB expression shortly following exposure and that these alterations become more subtle across the lifespan. Supported by NIH/NIGMS COBRE: The Delaware Center for Neuroscience Research grant 1P20GM103653 - 01A1 to AYK.

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Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA21311

Title: Gestational alcohol exposure dysregulates fetal brain iron homeostasis under maternal iron sufficiency and deficiency

Authors: S. M. HUEBNER[1], *P. D. DREW[2], S. M. SMITH[1];
[1]Nutritional Sci., Univ. of Wisconsin-Madison, Madison, WI; [2]Neurobio. and Developmental Sci., Univ. Arkansas Med. Sci., LITTLE ROCK, AR

Abstract: Prenatal alcohol exposure (PAE) impairs brain development, leading to lifelong deficits in IQ and executive function found in fetal alcohol spectrum disorders (FASD). Gestational iron deficiency (ID) mimics FASD's neurodevelopmental disabilities. We previously showed that maternal iron status influences FASD outcomes and dietary prevention of gestational maternal ID diminishes alcohol's developmental toxicity. In the non-pregnant adult, alcohol dysregulates iron homeostasis; the molecular and developmental consequences of iron dysregulation to fetal development are poorly understood. Using a 2nd trimester rat gestational PAE model, we studied the impact of PAE upon fetal iron homeostasis. Specifically, we measured the iron mineral content and homeostatic biomarkers of ferritin (FTN), transferrin (TF), transferrin receptor (TFRc), ferroportin (FPN), and divalent metal transporter 1 (DMT1), and hepcidin in fetal brain and liver under the conditions of maternal iron sufficiency (IS) and ID. Data collected indicate PAE inhibits the fetus from correctly interpreting maternal iron status, thus leading to interruptions in the proper homeostatic adaptations. PAE appears to disconnect signals that communicate iron status between fetal brain and liver. Under both IS and

ID, PAE increases fetal liver hepcidin, FTN expression and iron content at the expense of the brain. Moreover, data indicate that PAE further dysregulates the fetal brain's ability to compensate for ID, as evidenced by reductions in brain TF and TFRc expression. In contrast, a dysregulation of TF and TFRc expression is not observed in the IS alcohol-exposed fetus. With respect to fetal liver, PAE does not induce changes in TF and TFRc expression regardless of maternal iron status. As a whole, our data suggest that PAE during disrupts the fetal brain's ability to correctly adapt to maternal iron status, and these adaptive mechanisms are more dysregulated under maternal ID. This is a first step to build a detailed portrait of alcohol's effect upon iron homeostasis during gestation. Understanding the impact of maternal iron status during PAE will inform whether clinical intervention with maternal iron supplements will be efficacious in improving neurodevelopmental outcomes in FASD. Supported by AA21311.

Disclosures: S.M. Huebner: None. **P.D. Drew:** None. **S.M. Smith:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS R01MH069826.

Title: Bumetanide mitigates ethanol-induced aberrant migration of MGE-derived GABAergic interneurons in mouse embryonic dorsomedial telencephalon

Authors: J. T. WEISS, A. G. J. SKORPUT, N. M. SIMINERI, J. C. FAN, P. W. YEH, *H. H. YEH;
Physiol. and Neurobio., Geisel Sch. of Med. at Dartmouth, LEBANON, NH

Abstract: GABA, activating $GABA_A$ receptors, regulates corticopetal migration of GABAergic cortical interneurons during embryonic corticogenesis. This GABA-mediated migration is accelerated *in vivo* by prenatal exposure to ethanol, a canonical positive modulator of the $GABA_A$ receptor. $GABA_A$ receptor activation depolarizes these cells due to increased developmental expression of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1), maintaining $[Cl^-]_i$ higher than $[Cl^-]_e$. Based on this sequel of mechanisms, we hypothesized that ethanol, in potentiating $GABA_A$ receptor function, surges chloride efflux, augmenting the GABA-induced depolarization and, thereby, boosting and misguiding tangential migration. Here, we present experimental

results testing the corollary mechanistic hypothesis that the ethanol-induced aberrant tangential migration can be pharmacologically mitigated with bumetanide, an inhibitor of NKCC1 activity in the brain. Cre-recombinase expressed under control of the *Nkx2.1 promotor in the tdTomato reporter mouse (Nkx2.1-tdTomato)* yields intensely red-fluorescent MGE-derived, GABAergic interneurons. We found NKCC1-like immunoreactivity in cortical neurons as early as embryonic day(E) 14.5. On E14.5, 300-micron telencephalic slices were obtained from *Nkx2.1-tdTomato* embryos. The slices were divided into 4 groups: Control; 50mM Ethanol; 20 micromolar Bumetanide; 50mM Ethanol+20 micromolar Bumetanide. Ethanol and/or bumetanide were added to the slice medium at t_{1hr}. The media was replaced and replenished with drug at 6hr intervals. The slices were fixed at t_{25hr}, resectioned at 30μm, imaged, and the length of the cortex was divided into 100-μm bins extending from the corticostriate junction to the dorsal apex. The number of tdTomato-fluorescent cells was then analyzed for each group in a blinded fashion. Such analyses yielded significantly higher numbers of tdTomato-flourescent cells in slices exposed to ethanol, as reported *in vivo,* indicating a greater degree of MGE-derived tangential migration into the neocortex. The number of migrating cells dropped towards control levels in the group treated with ethanol+bumetanide, suggesting that bumetanide prevented the ethanol-induced heightening of tangential migration. The cell number was similar in slice cultures treated with and without bumetanide alone. Experiments are also evaluating the translatability of bumetanide treatment to assess prevention or rescue of aberrant tangential migration *in vivo* following ethanol exposure *in utero*.

Disclosures: J.T. Weiss: None. **A.G.J. Skorput:** None. **N.M. Simineri:** None. **J.C. Fan:** None. **P.W. Yeh:** None. **H.H. Yeh:** None.

Poster

712. Alcohol: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC 40076

Title: Paternal preconception alcohol exposure: Is there a risk to offspring?

Authors: *A. F. HARKER, S. RAZA, M. BEXTE, B. KOLB, R. GIBB;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: The effect of maternal experience on the prenatal environment is well researched, however, less is known about the paternal preconception influence on developing offspring. Maternal prenatal exposure to alcohol has been shown to be detrimental to offspring neurodevelopment, in fact, Fetal Alcohol Spectrum Disorder (FASD) is the leading known cause of developmental disabilities resulting from maternal exposure to alcohol during the prenatal period. As there is limited evidence regarding the heritable effects of preconception paternal exposure to alcohol, we examined the consequences of paternal alcohol on the developing brain and lifelong behaviour of offspring. Long Evans adult male rats were introduced to alcohol treatment starting at 2.5% ethanol-water solution followed by an increase of 2.5% ethanol concentration every other day, to a maximum of 20% ethanol concentration. This concentration was administered the remaining 34 days for a total of 48 days, which is the duration of one spermatogenic cycle in a rat. In order to examine the immediate effects of preconception paternal alcohol on developing offspring, males were mated with naïve Long Evans female rats immediately following cessation of alcohol treatment, resulting in the first cohort of offspring. In order to examine any prolonged effects of alcohol treatment in males, a second mating of the aforementioned mating pairs occurred 48 days (one spermatogenic cycle) following the cessation of alcohol treatment, producing the second cohort. Developmental, epigenetic and anatomical analyses, conducted throughout offspring lifespan, provided measures of paternal alcohol influence on offspring behaviour and neurodevelopment in both cohorts.

Disclosures: **A.F. Harker:** None. **S. Raza:** None. **M. Bexte:** None. **B. Kolb:** None. **R. Gibb:** None.

Poster

712. Alcohol: Developmental Effects

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Collaborative Initiative on Fetal Alcohol Spectrum Disorders

Title: Binge-like alcohol exposure early in gestation differentially alters stress reactivity in adult male and female mice

Authors: *L. A. WIECZOREK[1,2], E. W. FISH[1,2], S. E. PARNELL[1,2], K. K. SULIK[1,2];
[1]Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [2]Bowles Ctr. for Alcohol Studies, Chapel Hill, NC

Abstract: Alcohol use during pregnancy is one of the leading known causes of birth defects. While virtually all stages of prenatal development are vulnerable to alcohol-induced teratogenesis, insult at early developmental stages is especially concerning since 1) it often occurs before typical pregnancy recognition and 2) can cause serious and permanent brain damage. The current study used a mouse model of early prenatal alcohol exposure (PAE). Briefly, an acute, high-dose alcohol treatment (peak BAC ~420mg/dL) was given on gestational day (GD) 7 (3 week human equivalent) to pregnant dams and the resulting offspring (GD7 Alc mice) were analyzed for long-term consequences. This study examined the consequences of early PAE on stress reactivity in both male and female mice. Regarding measures of anxiety-like behavior, early PAE caused a sexually dimorphic response. Male GD7 Alc mice displayed increased anxiety-like behavior as measured by decreased time spent in the light side of a light-dark box. However, females were less anxious based on their increased number of entries into the light side of the box. The decreased anxiety-like phenotype in female GD7 Alc mice was further corroborated by them showing increased time in the open arms of an elevated plus maze. Molecular changes may account for some of the behavioral phenotype as male GD7 Alc mice displayed increased corticotrophin-releasing hormone receptor (CRHR) 1 mRNA in the pituitary, while female GD7 Alc mice displayed increased CRHR2 mRNA in the hippocampus. Based on knock-out studies, CRHR1 and CRHR2 have anxiogenic and anxiolytic properties, respectively. To further characterize early PAE's effect on stress reactivity, the hypothalamic-pituitary-adrenal (HPA) axis response was analyzed. Both male and female GD7 Alc mice showed a hyperreponsiveness of the HPA axis as evident immediately after a 15min restraint exposure by increased corticosterone (CORT) or ACTH, respectively. Despite having an increased CORT response maintained up to 90 min after stress exposure, ACTH returned to control levels by 60 min after stress exposure, resulting in an increased CORT:ACTH ratio. This may reflect enhanced negative feedback or continued hyperreponsiveness of the adrenals to ACTH release. Collectively, these data suggest that even a single PAE during early gestation can cause altered stress reactivity that is gender-specific and long lasting. To further advance our understanding of the functional changes occurring after acute stress exposure following early PAE, a manganese-enhanced magnetic resonance imaging approach is being applied to the study of this mouse model.

Disclosures: L.A. Wieczorek: None. **E.W. Fish:** None. **S.E. Parnell:** None. **K.K. Sulik:** None.

Poster

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Program#/Poster#: 712.18/AA7

Topic: C.17. Drugs of Abuse and Addiction

Title: Binge alcohol consumption causes alteration of gene expressions in the developing fetal brain

Authors: *K. JUNG, C. MANDAL, J. PARK, H. LEE, S. KIM, K. PARK, Y. CHAI;
Dept. of Mol. and Life Sci., Hanyang Univ., Ansan, Korea, Republic of

Abstract: Fetal alcohol spectrum disorder (FASD) is a set of developmental malformations in the nervous system induced by maternal alcohol consumption during pregnancy. The objective of the present study was to investigate the changes in gene expression in the fetal brain (forebrain and hippocampus) caused by maternal binge alcohol consumption. Pregnant C57BL/6J mice were treated intragastrically with distilled phosphate-buffered saline (PBS) or ethanol (2.9 g/kg) from embryonic day (ED) 8 to 12. Microarray analysis revealed that a significant number of genes were altered at ED13, ED15 and ED18 in the developing brain. Specifically, at ED18, nuclear factor one alpha (Nfia) and three N-methyl-D-aspartate (Nmda) receptors (Nmdar1, Nmdar2b and Nmdar2d) were down-regulated in the mouse fetal hippocampus. The transcription factor Nfia controls gliogenesis, cell proliferation and Nmda-induced neuronal survival by regulating the expression of target genes. We also found that the Nfia target genes Aldh1a, Folh1, Clu and Csrp1 showed changes in expression in the hippocampus under binge alcohol treatment, as expected. These results suggest that the altered expression of Nfia and Nmda receptors may be associated with the etiology of fetal alcohol syndrome (FAS). The data presented in this report will contribute to the understanding of the molecular mechanisms associated with the effects of alcohol in FASD individuals.

Disclosures: K. Jung: None. **C. Mandal:** None. **J. Park:** None. **H. Lee:** None. **S. Kim:** None. **K. Park:** None. **Y. Chai:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS R01MH069826

Title: *In utero* ethanol exposure alters MGE-derived interneuron distribution and inhibitory/excitatory balance in layer 5 pyramidal neurons of the mPFC

Authors: *A. G. SKORPUT, P. W. L. YEH, H. H. YEH;
Physiol. and Neurobio., Geisel school of medicine at Dartmouth, Lebanon, NH

Abstract: Fetal alcohol spectrum disorder presents with deficits in cortical function, notably the executive functions associated with the medial prefrontal cortex. Ethanol is a canonical positive allosteric modulator of the $GABA_A$ receptor. Since $GABA_A$ receptor mediated signaling impacts many aspects of corticogenesis (neurogenesis, migration), they may be particularly susceptible to the effects of *in utero* ethanol exposure at a gestational stage when neurogenesis and neuronal migration is at their height. In addition, since proper cortical circuit function relies on the establishment of appropriate balance between inhibition and excitation (I/E balance) of cortical neurons, one functional consequence of a prenatal ethanol exposure-induced aberrant neuronal migration may be a shift from the normal I/E balance in cortical neurons. To test this hypothesis, we assessed I/E balance in layer 5 cortical pyramidal neurons that serve as the principal cortical efferent system. Here we report altered distribution of mPFC GABAergic interneurons derived from the medial ganglionic eminence (MGE) following chronic or binge *in utero* ethanol exposure, modeled via maternal consumption of a liquid diet containing 2% EtOH from E9.5-birth or 5% EtOH from E13.5-E15.5, respectively. Chronic exposure resulted in increased BrdU labeling in the MGE, consistent with an increase in the number of parvalbumin positive (PV+) interneurons in the mPFC of adult offspring. Binge exposure resulted in increased counts of migrating Nkx2.1 lineage neuroblasts in the embryonic dorsomedial telencephalon, and a commensurate increase in the number of PV+ interneurons in the mPFC of adult offspring. In addition, we report altered I/E balance in layer 5 pyramidal neurons of the mPFC. Chronically exposed offspring exhibited increased spontaneous I/E ratio due to a reduction in sEPSC frequency. Binge exposure, on the other hand resulted in increased sIPSC frequency; the result of this on I/E balance is currently being investigated. Our models of chronic and binge *in utero* ethanol exposure both resulted in offspring with a hyperactive phenotype. Furthermore, binge-exposed mice tested in the modified Barnes maze exhibited a deficit in their ability to efficiently navigate the maze, particularly during reversal trials that critically depend on proper mPFC function. Overall, we conclude that *in utero* ethanol exposure leads to enduring changes in cortical form and function in the offspring, as assessed here in the mPFC, and that these changes contribute to mPFC-dependent behavioral deficits later in life.

Disclosures: **A.G. Skorput:** None. **P.W.L. Yeh:** None. **H.H. Yeh:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: AA019462

AA019884

Title: Enhanced NMDA-GluN2B subunit expression in rat ventrolateral frontal cortex following moderate prenatal ethanol exposure

Authors: ***C. W. BIRD**[1], F. CANDELARIA-COOK[1], C. MAGCALAS[1], S. DAVIES[2], D. SAVAGE[2], F. VALENZUELA[2], D. HAMILTON[1,2];
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Abstract: Heavy ethanol consumption during pregnancy leads to fetal alcohol syndrome in offspring, characterized by gross morphological abnormalities and cognitive deficits. More limited ethanol exposure during gestation results in more subtle neurobiologic and behavioral changes, which are described under the umbrella term fetal alcohol spectrum disorders (FASD). Characterizing the teratogenic effects of moderate ethanol consumption by pregnant mothers will be crucial to developing treatments for improving cognition in affected individuals. Prenatal ethanol exposure has been shown to alter the composition of glutamatergic NMDA receptors. During normal development, the subunit composition of NMDA receptors switches from containing mostly GluN2B subunits to containing predominantly GluN2A subunits. GluN2A and GluN2B containing NMDA receptors have different channel kinetics, which can affect long term potentiation as well as other neuronal properties. Pregnant Long-Evans rats were exposed to either saccharin or ethanol during gestation using a voluntary exposure paradigm which results in moderate drinking. GluN2B subunit expression was measured in adult offspring using a combination of radioligand binding studies and electrophysiology. Specific [3H]-ifenprodil binding to GluN2B subunit-containing NMDA receptors was increased in cortical layers II/III of the agranular insular cortex (AID) but not other frontal regions of rats exposed to ethanol during gestation. To further evaluate this finding, evoked NMDA receptor-mediated excitatory

postsynaptic currents (NMDAR-EPSCs) were measured in pyramidal layer II/III neurons from the AID in the whole-cell patch-clamp configuration to quantify sensitivity to the selective negative allosteric modulator of GluN2B subunit-containing receptors, ifenprodil. Three μM ifenprodil caused a greater reduction in NMDAR-EPSCs from fetal ethanol-exposed animals than saccharin control offspring, consistent with an increased density of GluN2B containing NMDA receptors in the AID region of ventrolateral frontal cortex. Together, these data indicate that moderate prenatal ethanol exposure has a significant impact on excitatory neurotransmission in the frontal cortex, which could have functional consequences impacting social behavior, behavioral flexibility, motor control and other behavioral and cognitive processes that require and engage AID and related ventrolateral frontal cortical regions.

Disclosures: C.W. Bird: None. **F. Candelaria-Cook:** None. **C. Magcalas:** None. **S. Davies:** None. **D. Savage:** None. **F. Valenzuela:** None. **D. Hamilton:** None.

Poster

712. Alcohol: Developmental Effects

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 712.21/AA10

Topic: C.17. Drugs of Abuse and Addiction

Support: R37 AA08757

Title: Opioid agonist and ethanol induce microglial activation via TLR-2/4 mediation and promotes cytokines induced neurotoxicity in hypothalamic microglial culture

Authors: P. SHRIVASTAVA, *D. K. SARKAR;
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Abstract: Microglia cells play pivotal role in neuroinflammatory and immune responses in the brain and have been shown to play a role in the mechanisms of ethanol-induced killing of neurons in the hypothalamus. We also recently found that alcohol-induced neuroinflammatory response of microglia might involve interaction with the opioid receptors. The present study was designed to investigate further the interactions of opioid receptor and ethanol in hypothalamic microglia *in vitro*. We used one-day old SD rat pups to isolate and prepare hypothalamic microglia cell cultures. Microglia cell cultures were treated with vehicle, ethanol with or without μ-opioid receptor agonist DAMGO for a period of 24 h. We then used these cell cultures for determination of changes in the Toll-like receptors (TLRs) levels on microglial cells and also to

determine the microglia activation marker IBA1 protein level. Opioid agonist DAMGO (50μM) and ethanol (50mM) treatment increased the expression of TLR-2 transcripts, whereas the TLR-4 expression was significantly increased only following the combination of DAMGO and ethanol. In addition, ethanol and DAMGO treatments augmented TNF-α mRNA expression. Moreover, IBA-1 protein levels were elevated in ethanol and DAMGO treated microglia, suggesting microglial activation. These results suggest the possibility that TLR2/4 receptors may be involved in ethanol activation of microglia and thereby neuroinflammation processes.

Disclosures: P. Shrivastava: None. **D.K. Sarkar:** None.

Poster

712. Alcohol: Developmental Effects

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 712.22/AA11

Topic: C.17. Drugs of Abuse and Addiction

Title: Dysregulation of ILK signalling is associated with hippocampal based memory and synaptic plasticity in FASD rat model

Authors: *D. BHATTACHARYA[1], E. DUNAWAY[2], J. BLOEMER[1], S. BHATTACHARYA[1], M. BUABEID[1], M. ESCOBAR[2], V. SUPPIRAMANIAM[1], M. DHANASEKARAN[1];
[1]Dept. of Drug Discovery and Develop., [2]Dept. of Psychology, Auburn Univ., Auburn, AL

Abstract: Introduction: Drinking alcohol during pregnancy leads to a range of psychological and physiological defects in children, which can be grouped under the term Fetal Alcohol Spectrum Disorder (FASD). According to the Centre of Disease Control and Prevention, FASD has a prevalence of 1.5-2.0 cases per 1000 births in the US. To date, there is no treatment for FASD, but some of its effects (e.g., memory deficits and decreased synaptic plasticity) are well characterized. Integrin Linked Kinase (ILK) signalling plays a significant role in memory and synaptic plasticity. ILK signalling regulation could be critical in neuronal deficits. In FASD, Glycogen Synthase Kinase 3β (GSK3β), downstream to ILK remains active. Therefore, we hypothesize that prenatal alcohol impairs ILK signalling mediated synaptic plasticity and behaviour. Methods: Pregnant Sprague Dawley rats consumed alcohol (2.5% to 10% v/v) throughout gestation. On postnatal days 32-33, the pups were exposed to a novel environment which was paired with a mild aversive stimulus, and memory of the dangerous nature of the environment was assessed 24 h later. Synaptic plasticity was assessed by measuring hippocampal field potentials in Schaffer Collateral and hippocampal protein lysates were used to evaluate ILK

signalling and activity. Protein complex immunoprecipitation was used to assess the interaction of ILK with the receptors and trafficking proteins. Results: Pups exposed to alcohol prenatally showed very little memory of the aversive event as compared to control pups that received no alcohol exposure, suggesting that prenatal alcohol exposure causes hippocampal memory impairment. This was confirmed by the observation of a 30% decrease in LTP in alcohol-exposed but not control pups. Western blot analyses also showed significant reduction in GSK3β (Ser21/9) phosphorylation which is downstream to ILK signalling pathway. Reduced phosphorylation or active GSK3β has been previously shown to impair memory and plasticity. ILK activity but not expression was reduced in alcohol-exposed pups. Interaction of ILK with glutamate receptors (NMDAR and AMPAR subunits) and trafficking molecules such as stargazin was also impaired. Conclusion: ILK signalling plays a significant role in impaired memory and plasticity related to FASD. In future, activating the ILK pathway through intracranial BDNF infusion will be assessed in FASD rat model to further validate our hypothesis.

Disclosures: D. Bhattacharya: None. **E. Dunaway:** None. **J. Bloemer:** None. **S. Bhattacharya:** None. **M. Buabeid:** None. **M. Escobar:** None. **V. Suppiramaniam:** None. **M. Dhanasekaran:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.01/AA12

Topic: C.19. Drug Discovery and Development

Title: Recombinant human serum albumin fused with human butyrylcholinesterase (TV-1380) as a bioscavenger of organophosphorus nerve agents

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Abstract: Nerve agents (NA) are highly lethal chemicals, of which many belong to the organophosphorus (OP) compound group; they are among the most toxic substances identified. Human butyrylcholinesterase (BChE) is a naturally occurring serum protein that rapidly and irreversibly binds OPs, preventing toxicity. TV-1380 is a recombinant human serum albumin (HSA)-fused to a variant of human butyrylcholinesterase (BChE) which is currently in Phase 2 clinical trials for use as a cocaine-hydrolyzing enzyme. TV-1380 was tested for the ability to sequester free OP nerve agents both *in vitro* and in preclinical animal models. Isothermal titration calorimetry was used to determine Michaelis-Menten kinetic constants, and a continuous activity assay was used to measure inhibition rate constants with the OP nerve agents tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), VX, Russian VX (VR), and VM. Spontaneous and oxime-mediated reactivation rates with oximes 2PAM, MMB4 and HI6 were also measured. Protective efficacy studies in guinea pigs (GPs) were conducted using TV-1380 injected iv at 50 mg/kg and OP agents administered sc at twice the median lethal dose 30 minutes post enzyme injection. TV-1380 had a six-fold lower hydrolysis rate against BTCh than WT BChE, with both displaying near linear (R^2>0.99) concentration/activity relationships. TV-1380 hydrolysis of BTCh as measured by isothermal titration calorimetry determined a K_M of 5.36 μM with a K_i of 386.4 μM, indicative of substrate inhibition. Inhibition rate constants ($x10^5$ M^{-1} min^{-1}) for TV-1380 were determined to be 6.12 (GA), 10.3 (GB), 109 (GD), 203 (GF), 0.398 (VX), 0.339 (VR), and 17.8 (VM) compared to WT BChE at 49.3(GA), 86.2 (GB), 852 (GD), 1610 (GF), 69.2 (VX), 26.2 (VR), and 1080 (VM). No spontaneous reactivation was observed for TV-1380 after inhibition by OP nerve agents. No clinically significant half-times of reactivation ($t_{1/2}$) were observed using oxime reactivators. Protection studies showed 100% survival against GB, GD, GF and VR and 50% survival against GA for 50 mg/kg pretreatment of TV-1380 iv At 50 mg/kg of TV-1380, no protection against VX was observed, although 100% survival against VX was observed in animals that received a higher dose (267 mg/kg) of enzyme. These data show that TV-1380 can act as a stoichiometric nerve agent bioscavenger by binding various OP agents in circulation and preventing them from interacting with AChE. Until other bioscavenger agents are developed with longer protection periods, TV-1380 would be a cost-effective, short-acting option to protect against OP nerve agents.

Disclosures: **M.M. Seavey:** A. Employment/Salary (full or part-time):; Teva Pharmaceuticals USA. **D.M. Cerasoli:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva Pharmaceuticals. **C.L. Cadieux:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva Pharmaceuticals. **M.V. Boeri:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva Pharmaceuticals. **S.M. Hodgins:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva Pharamceuticals. **C.A. Hofstetter:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva pharmaceuticals. **S.A. Kasten:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva pharmaceuticals. **T.C. Otto:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teva pharmaceuticals. **H. Hallak:** A.

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Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.02/AA13

Topic: C.19. Drug Discovery and Development

Title: Development of new peptide vector family, Transcend (melanotransferrin-MTf, p97) first and second generation, for the delivery of biologics across the Blood-Brain Barrier in the brain

Authors: *R. GABATHULER[1], M.-M. TIAN[1], T. ABRAHAM[2], M. I. NOUNOU[3], P. R. LOCKMAN[4], W. JEFFERIES[5];
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Abstract: The blood-brain barrier (BBB) is formed by brain capillary endothelial cells characterized by tight junctions between cells and a high expression of efflux pumps only allowing brain access to nutrients necessary for cell survival and function. These properties of the BBB result in the incapacity of small and large therapeutic compounds to reach the brain in therapeutic concentrations. Biologics such as Trastuzumab, a humanized monoclonal antibody, recognizing HER2 improves survival of patients with metastatic breast cancer that overexpress HER2. However, when patients with metastatic breast cancer are treated with Trastuzumab, the breast cancer frequently progresses in the CNS due to the poor penetration of antibodies into the brain. A vector family called Transcend comprising a full length protein (Melanotransferrin-MTf, p97) and peptide originating from Transcend (MTf) have been developed by biOasis Technologies Inc. and used in receptor mediated drug delivery into the brain to treat CNS

disorders. In a study to extend its application to biologics, MTf has been conjugated to Tratuzumab labeled with a fluorescent marker and its uptake in-vitro in cells in culture and in-vivo in mice brains determined. We can clearly detect binding of Trastuzumab and its conjugate to MTf (BT2111) to the cell surface and uptake in BT474 and able to recognize its target molecule. In mice, using marker proteins labelled with fluorescent dyes that bind to molecules expressed specifically on brain capillary endothelial cells the quantification of proteins localized in the brain parenchyma was possible. MTf has demonstrated that antibodies labelled with rhodamine or other fluorescent dyes can be transported in the brain parenchyma after its incorporation. By quantitative confocal fluorescence microscopy we determined that 10 to 15 times more antibodies were delivered in the brain parenchyma when conjugated to MTf. Transcend when incorporated in Trastuzumab is homogenously distributed in normal brain and reduces by 68% the number and by 57% the size of the remaining metastases in the brain when compared to Trastuzumab treated animals. In addition to Transcend, we will show that a family of peptides originating from the Transcend protein (MTf) can transport with a high rate a variety of therapeutic biologics across the BBB and demonstrate that they can be used as vector for the transport of therapeutics across the BBB for the treatment of neurological disorders. In addition to deliver therapeutic compounds in the brain parenchyma MTf and its derived peptide family do deliver these compounds to specific brain cells and to their intracellular compartment likely endosomes and lysosomes.

Disclosures: **R. Gabathuler:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); biOasis Technologies Inc.. F. Consulting Fees (e.g., advisory boards); biOasis Technologies Inc. **M. Tian:** F. Consulting Fees (e.g., advisory boards); biOasis Technologies inc.. **T. Abraham:** None. **M.I. Nounou:** None. **P.R. Lockman:** None. **W. Jefferies:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); biOasis Technologies Inc.. F. Consulting Fees (e.g., advisory boards); biOasis Technologies inc..

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.03/AA14

Topic: C.19. Drug Discovery and Development

Title: *In vitro* characterization of LY3020371, a potent and selective mGlu2/3 receptor antagonist

Authors: *J. A. MONN[1], J. M. WITKIN[1], P. L. ORNSTEIN[1], C. H. MITCH[1], R. LI[1], S. C. SMITH[1], X. WANG[1], C. XIANG[1], J. H. CARTER[1], J. WANG[2], S. ATWELL[2], F. PASQUI[3], S. M. FITZJOHN[3], B. A. HEINZ[1];
[1]Eli Lilly & Co., Indianapolis, IN; [2]Eli Lilly & Co., San Diego, CA; [3]Eli Lilly & Co., Windlesham, United Kingdom

Abstract: Small molecule modulators of glutamate neurotransmission continue to be of interest as therapeutic agents for the treatment of conditions for which aberrant glutamate signaling has been implicated. Metabotropic glutamate 2/3 (mGlu2/3) receptors have been of considerable interest in the field owing to their role in modulating glutamate transmission via presynaptic, postsynaptic and glial mechanisms. As part of our ongoing efforts to identify novel ligands for these receptors, we have discovered LY3020371, a potent and selective orthosteric mGlu2/3 antagonist. In membranes from cells expressing recombinant human mGlu2 and mGlu3 receptor subtypes, LY3020371 competitively displaced high affinity [^{3}H]-459477 radioligand binding (K_i = 3.54 ± 0.53 nM for mGlu2 and 2.32 ± 0.41 nM for mGlu3) while in cells expressing these receptors, LY3020371 demonstrated competitive and full reversal of DCG-IV-inhibited, forskolin-stimulated cAMP formation (mGlu2 IC_{50} = 15.4 ± 2.04 nM; mGlu3 IC_{50} = 6.21 ± 2.15 nM). Evaluation of LY3020371 in cells expressing the other human mGlu receptor subtypes as well as a panel of over seventy additional CNS receptors, transporters, ion channels and enzymes, revealed high mGlu2/3 receptor selectivity. Further characterization of the binding of LY3020371 to the glutamate binding site of hmGlu2 was established by co-crystallization of this molecule with the amino terminal domain of the mGlu2 receptor. The resulting co-crystal structure revealed the specific ligand-protein interactions which likely lead to the high affinity of LY3020371 for this site and supported its functional mGlu2 antagonist activity. In rat native tissue assays, LY3020371 demonstrated effective displacement of [^{3}H]-459477 from rat frontal cortical membranes, and functional antagonist activity as measured by reversal of mGlu2/3 agonist (LY379268) - inhibited, forskolin-stimulated cAMP in rat cortical synaptosomes. To confirm a functional mGlu2/3 receptor antagonist effect on synaptic transmission, the effect of LY3020371 on mGlu2/3 agonist-inhibited field evoked excitatory post synaptic potentials (fEPSPs) in an intact hippocampal slice preparation (MPP-DG synapse) was characterized. In this assay, compound LY3020371 displayed a concentration-dependent reversal of LY379268-inhibited fEPSPs with a calculated IC_{50} of 46 nM. LY3020371 exhibited high aqueous solubility, low passive permeability, low microsomal metabolism and low plasma protein binding. LY3020371 is therefore established as a useful tool for studying mGlu2/3R *in vitro* and may be a competent agent for interrogating these targets *in vivo* (see Witkin et al. abstract, this meeting).

Disclosures: **J.A. Monn:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **J.M. Witkin:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **P.L. Ornstein:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **C.H. Mitch:** A.

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Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.04/AA15

Topic: C.19. Drug Discovery and Development

Support: NHMRC-APP1058051

Title: Vascular targeting for new treatment of brain arteriovenous malformation

Authors: *S. UGOYA[1], Z. ZHAO[2], M. STOODLEY[2];
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Abstract: Arteriovenous malformations (AVMs) of the brain are devastating congenital lesions that are the most common cause of haemorrhagic stroke in children and young adults. Although most small AVMs are curable, over 90% of large lesions are untreatable with current techniques. Our goal is to develop a new treatment for AVMs, using pro-thrombotic agents to occlude AVM vessels, thereby eliminating the risk of brain haemorrhage. Pro-thrombotic agents are created by conjugating a thrombotic molecule (such as tissue factor or thrombin) with a ligand (such as an antibody) directed against an endothelial target. Vascular targeting is conceptually very attractive for the treatment of AVMs: the goal is purely vascular occlusion and not cell death. However, the technique must be precise, as inadvertent occlusion of vessels supplying normal brain is potentially catastrophic. To induce selective occlusion of AVM vessels, an endothelial surface molecule that is highly discriminating between AVM vessels and normal vessels is required. Shear stress induction is said to regulate endothelial cell (EC) activities and behavior and

modulates several gene expressions via the shear stress response element (SSRE). In order to understand vascular targeting, we utilized *in vitro* thrombus methods formation adapted with flow chamber-based devices (Glycotech™), which uses whole blood perfusion pumps that mimics wall shear stress (WSS). These pumps are fitted with device that regulates laminar shear stress and can systematically adjust the WSS to desired level which can be analyzed with tracking software. We have been able to determine and quantify the shear stress using fluorescent tracer particles which also showed increased shear stress at high flow rate of 8-30dyne/cm2. We have successfully labeled human platelets with DIOC6 and R-6-G and were able to show differential binding and tethering of platelets both at high and low pump speed. We have developed some conjugates and still testing the efficacy and thrombus stability at different WSS. We are still testing this model in our rat AVM model and this look promising. Vascular targeting may be a potential treatment for most untreatable BAVMs.

Disclosures: S. Ugoya: None. **Z. Zhao:** None. **M. Stoodley:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.05/AA16

Topic: C.19. Drug Discovery and Development

Title: Careful consideration of the difference in comedications can substantially improve success in CNS clinical trials

Authors: *H. A. GEERTS[1], A. SPIROS[2];
[1]In Silico Biosci., Berwyn, PA; [2]In Silico Biosci., Portland, OR

Abstract: Success rates in CNS Clinical Trials have not improved despite substantial increased understanding of the disease neurobiology. While this failure rate might be due to mismatched target, the wrong patient population, insufficient target engagement or mismatch between rodent and human pharmacology, we will focus on the interference of comedications in clinical trials. Many approved CNS active drugs do have a rich pharmacology and can affect neuronal circuits in non-linear ways. In addition, many patients are on a number of comedications. We use an advanced version of a computer-based Quantitative Systems Pharmacology (QSP) platform, a mechanism-based computer model of the relevant humanized cortical networks that has been developed for clinical readouts in psychiatry and neurology and has been calibrated with group average clinical data. The platform has been able to blindly predict an unexpected clinical

outcome in three occasions. By implementing the human pharmacology of known comedications, we can simulate the impact of comedications on dose-responses for a number of single targets. Histamine H3 antagonism improves the outcome for cognitive impairment in schizophrenia when added to olanzapine but not to risperidone. The dose-response of an alpha-7 nicotinic receptor agonist in cognitive impairment in schizophrenia is affected by the type of antipsychotic given as comedication; risperidone, olanzapine, quetiapine and clozapine increase the level of free ACh to different degrees, while haloperidol and aripiprazole do not have any effect. In addition, the effect of an alpha4beta2 nAChR modulator is also modulated by smoking state. In AD, H3 antagonism improves the cognitive outcome in AD at earlier time points (4-12 weeks), but not at 26 weeks, an effect amplified by the presence of an AChE-I such as donepezil. Antidepressants differentially effect the cognition in AD as they affect the basal level of 5-HT and modify the cognitive status through 5-HT3, 5-HT4 and 5-HT6 receptors. These results suggest that each CNS active comedication has a complex pharmacology and can affect the dose-response of single target drugs differentially. The study also suggests that the traditional concept of chlorpromazine equivalents for antipsychotic drug treatment has severe limitations, as the particular drug pharmacology fingerprint for each drug against human receptors has a very unique impact. Simulating virtual patients using a QSP-based approach is a way to identify possible comedications that need to be excluded or balanced across different treatment arms in order to significantly increase the probability of success.

Disclosures: H.A. Geerts: A. Employment/Salary (full or part-time):; In Silico Biosciences. **A. Spiros:** A. Employment/Salary (full or part-time):; In Silico Biosciences.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.06/AA17

Topic: C.19. Drug Discovery and Development

Title: Characterization of binding and inhibitory properties of TAK-063, a novel PDE10A inhibitor

Authors: *A. HARADA, K. SUZUKI, J. KUNITOMO, N. KAMIGUCHI, M. MIYAMOTO, K. TOHYAMA, K. NAKASHIMA, T. TANIGUCHI, H. KIMURA;
Takeda Pharmaceut. Co., Fujisawa / Kanagawa, Japan

Abstract: Phosphodiesterase 10A (PDE10A) inhibition could be a novel approach for the treatment of disorders such as schizophrenia. A novel PDE10A inhibitor, TAK-063, showed high inhibitory activity and selectivity for human recombinant PDE10A2 *in vitro*; IC_{50} was 0.30 nmol/L, and selectivity over other PDEs was more than 15,000-fold. TAK-063 at 10 μmol/L did not show more than 50% inhibition or stimulation for 91 enzymes and receptors except for PDEs. *In vitro* autoradiography (ARG) using rat brain sections revealed that [^{3}H]TAK-063 selectively accumulated in caudate putamen (CPu), nucleus accumbens (NAc), globus pallidus, substantia nigra, and striatonigral projection, where PDE10A is highly expressed. This [^{3}H]TAK-063 accumulation was mostly blocked by an excess amount of MP-10, a PDE10A selective inhibitor. This selective accumulation was not observed in brain slices of *Pde10a*-knockout mice. In rat brain sections, [^{3}H]TAK-063 bound to a single high affinity site with the K_d values of 7.2 ± 1.2 and 2.6 ± 0.5 nmol/L for CPu and NAc shell, respectively. In *in vivo* ARG study using a rat, orally administered [^{14}C]TAK-063 selectively accumulated in the same brain regions as observed in *in vitro* ARG. Striatal PDE10A occupancy of TAK-063 in rats was measured by using T-773 as a tracer. The dose of 0.88 mg/kg (p.o.) of TAK-063 was estimated to produce 50% occupancy level. Clinical and preclinical studies of TAK-063 based on such information will enable us to evaluate potentials of PDE10A inhibitors as drugs for several CNS disorders more precisely.

Disclosures: **A. Harada:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company. **K. Suzuki:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company. **J. Kunitomo:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company. **N. Kamiguchi:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company. **M. Miyamoto:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company. **K. Tohyama:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company. **K. Nakashima:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company. **T. Taniguchi:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company. **H. Kimura:** A. Employment/Salary (full or part-time):; Takdeda Pharmaceutical Company.

Poster

713. Drug Discovery and Delivery

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Program#/Poster#: 713.07/AA18

Topic: C.19. Drug Discovery and Development

Support: National Institute of Mental Health Psychoactive Drug Screening Program

Title: Pharmacological characterization and SAR studies of small molecular agonists for GPR88

Authors: *C. JIN[1], A. M. DECKER[1], X.-P. HUANG[2], B. P. GILMOUR[1], B. E. BLOUGH[1], B. L. ROTH[2];
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Abstract: GPR88 is an orphan G-protein-coupled receptor (GPCR) highly expressed in both dopamine D_1 and D_2 receptor-expressing medium spiny neurons. Genetic deletion and gene expression studies have suggested that GPR88 plays an important role in the regulation of striatal functions and is implicated in basal ganglia-associated disorders. However, the signal transduction pathway and receptor functions of GPR88 are still largely unknown due to the lack of endogenous and potent, selective synthetic ligands. Recently, a series of surrogate ligands have been implicated in GPR88 mediated activation of Gαi proteins. To develop an appropriate cell based assay system to support drug discovery for the GPR88 receptor, we synthesized a putative GPR88 agonist 2-PCCA and functionally characterized it in HEK293 cells both transiently and stably expressing GPR88. 2-PCCA inhibited isoproterenol-stimulated cAMP accumulation in a concentration-dependent manner in cells expressing GPR88 but not in the control cells, indicating that GPR88 is coupled to Gαi proteins. The assay provides a robust platform compatible for high throughput screening to identify potential ligands for the GPR88 receptor. Receptor specificity and structure-activity relationship (SAR) studies of 2-PCCA are also presented.

Disclosures: C. Jin: None. **A.M. Decker:** None. **X. Huang:** None. **B.P. Gilmour:** None. **B.E. Blough:** None. **B.L. Roth:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.08/AA19

Topic: C.19. Drug Discovery and Development

Title: Design and synthesis of TAK-063, a novel phosphodiesterase 10A (PDE10A) inhibitor for the treatment of schizophrenia

Authors: *J. KUNITOMO, M. FUSHIMI, M. YOSHIKAWA, A. KAWADA, T. HASUI, T. HITAKA, H. OKI, Y. HAYANO, H. KOKUBO, K. NAKASHIMA, M. KONDO, K. SUZUKI,

H. KIMURA, T. TANIGUCHI;
Takeda Pharmaceut. Co. Limited, Fujisawa / Kanagawa, Japan

Abstract: Phosphodiesterases (PDEs) are intracellular enzymes involved in the hydrolysis of cAMP and/or cGMP into their respective nucleotide monophosphates. Phosphodiesterase 10A (PDE10A) is expressed at high levels exclusively in the striatal medium spiny neurons. This tissue distribution pattern indicates that selective inhibition of PDE10A could be used to raise levels of cAMP and/or cGMP within cells and, therefore, would be useful in treating a variety of neuropsychiatric conditions, such as schizophrenia. High throughput screening (HTS) of our in-house chemical library identified a pyridazin-4(1H)-one derivative with a PDE10A IC50 = 23 nM and 110-fold selectivity against other PDEs. Our optimization efforts using SBDD based on the X-ray crystal structure of the hit compound with PDE10A led to the discovery of TAK-063 with markedly potent inhibitory activity (IC50 = 0.30 nM), excellent selectivity (more than 15,000-fold selectivity against other PDEs). TAK-063 exhibited good oral absorption and excellent brain penetration in mice, and also produced dose-dependent suppression of PCP-induced hyperlocomotion with a minimum effective dose of 0.3 mg/kg by oral administration in mice. Oral administration of TAK-063 to mice at a dose of 0.3 mg/kg caused increases in the striatal cAMP and cGMP. TAK-063 is currently under evaluation in Phase I clinical trials for the treatment of schizophrenia (ClinicalTrials.gov Identifiers: NCT01879722 and NCT01892189).

Disclosures: **J. Kunitomo:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Fushimi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Yoshikawa:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **A. Kawada:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **T. Hasui:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **T. Hitaka:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **H. Oki:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **Y. Hayano:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **H. Kokubo:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **K. Nakashima:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **M. Kondo:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **K. Suzuki:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **H. Kimura:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **T. Taniguchi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.09/AA20

Topic: C.19. Drug Discovery and Development

Support: Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan

Title: Encapsulated cell transplantation for central nervous system disorders

Authors: *T. YASUHARA, M. KAMEDA, T. AGARI, A. TOYOSHIMA, H. TAKEUCHI, J. MORIMOTO, A. KONDO, A. SHINKO, S. SASADA, T. SASAKI, T. WAKAMORI, M. OKAZAKI, I. DATE;
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Abstract: Encapsulated cell transplantation is one of good therapeutic tools for central nervous system disorders, such as Parkinson's disease and cerebral infarct in basic research. The characteristics of encapsulated cell transplantation are as follows. 1. Various neurotransmitters like dopamine, neurotrophic factors including glial cell line-derived neurotrophic factor (GDNF), or growth factors, such as vascular endothelial growth factor (VEGF) can be produced continuously from encapsulated cells with engineered properties. Cells inside the capsule are supplied sufficient nutrient and oxygen through the semipermeable membrane. 2. The capsule can be removed from the transplanted brain if need to be. 3. Scant immune reactions and no immunological rejection arise because cells inside are protected by the capsule. Additionally, there are no risks of tumor formation in the host tissue. 4. Various cells including immortalized cell lines can be transplanted safely as a surviving donor with no ethical problems. These cells are also engineered genetically with ease. 5. Encapsulated cell transplantation enables us to deliver continuous and low dose administration of secreting factors to the surrounding host brain, even in the case that the half life of the secreted molecule is extremely short. In this presentation, first we demonstrate encapsulated neurotrophic factor-secreting cell transplantation for cerebral ischemia model of rats. Second, encapsulated catecholamine-secreting cell controlled by Tet-off system for Parkinson's disease model of rats is focused. Third, our strategy using encapsulated stem cell transplantation is described to reveal the mechanisms of therapeutic effects of stem cell transplantation. Encapsulated cell transplantation might be a hopeful therapeutic tool in clinical settings as well as a useful technique in basic research.

Disclosures: T. Yasuhara: None. **M. Kameda:** None. **T. Agari:** None. **A. Toyoshima:** None. **H. Takeuchi:** None. **J. Morimoto:** None. **A. Kondo:** None. **A. Shinko:** None. **S. Sasada:** None. **T. Wakamori:** None. **M. Okazaki:** None. **I. Date:** None. **T. Sasaki:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.10/AA21

Topic: C.19. Drug Discovery and Development

Title: LY3020371 is a potent and selective competitive antagonist of mglu2/3 receptors *in vivo*

Authors: *J. M. WITKIN[1,2], C. OVERSHINER[2], X. LI[3], G. GILMOUR[3], J. LI[3], L. RORICK-KEHN[3], K. RASMUSSEN[3], B. JOHNSON[3], S. N. MITCHELL[3], D. MCKINZIE[3], A. NIKOLAYEV[3], V. TOLSTIKOV[3], M.-S. KUO[3], P. ORNSTEIN[3], C. MITCH[3], R. LI[3], S. SMITH[3], X.-S. WANG[3], B. HEINZ[3], D. ALLEN[3], S. SWANSON[3], J. MONN[3];
[1]Lilly Co Ctr., INDIANAPOLIS, IN; [2]Eli Lilly and Co., Indianapolis, IN; [3]Eli Lilly and Co, Indianapolis, IN

Abstract: Experimental investigations over a number of years have increasingly supported the potential antidepressant efficacy of mGlu2/3 receptor antagonists [1]. A replication of Berman and colleagues initial report [2] by Zarate and colleagues [3] on the efficacy of ketamine led to increased confidence that glutamate enhancing agents might be therapeutically valuable for treatment-resistant depressed patients [4]. We designed and synthesized a potent and selective bicyclohexane mGlu2/3 receptor antagonist (LY3020371) for biological profiling with this therapeutic indication in mind. Functional *in vitro* activity assays demonstrated strong correspondence from recombinant human receptors to native rat brain tissues [see Monn et al., this meeting]. *In vivo*, LY3020371 and ketamine engendered a host of overlapping biological activities: both compounds increased the number of spontaneously active DA cells in the ventral tegmental area of anesthetized rats, increased O_2 availability in the anterior cingulate cortex, promoted wakefulness, enhanced the efflux of biogenic amines in the prefrontal cortex, and produced antidepressant-related behavioral effects in SSRI-sensitive and SSRI-insensitive rodent models. Target specificity of LY3020371 was demonstrated via pharmacological and receptor knock-out mice studies. LY3020371 and ketamine both produced effects that were sensitive to AMPA receptor blockade and further implicated in metabolomic pathway analyses. In contrast to ketamine, LY3020371 did not increase dopamine efflux in the Nucleus Accumbens of rats (a marker for drug dependence), disrupt cognitive performances, or produce motor incoordination. LY3020371 is a highly soluble amino diacid. Clearance was low in both rats and monkeys after intravenous dosing. Single dose pharmacokinetics (i.v.) were linear in both rats (0.5-1000 mg/kg) and non-human primates (1-1000 mg/kg). Renal elimination was the only route of clearance observed; no metabolism of LY3020371 was detected by LC/MS/MS *in vitro* or *in vivo*. Brain

penetration achieved CSF exposures ranging from 2-6% of plasma values. Peak CSF concentrations exceeded the mGlu2 and mGlu3 *in vitro* IC_{50} at doses ≥ 1 mg/kg, i.v. There is a low probability of CYP-related drug-drug interactions for LY3020371 since it is not a substrate, inhibitor, or inducer of the CYP's examined. References [1] Pilc A et al. (2008) *Biochem Pharmacol* 75: 977-1006. [2] Berman RM et al. (2000) *Biol Psychiatry* 47: 351-354. [3] Zarate CA et al. (2006) *Arch Gen Psychiatry 63:* 856-64. [4] Witkin JM (2011) *CNS Neurol Disorders Drug Targets 10*: 1-2.

Disclosures: J.M. Witkin: None. **C. Overshiner:** A. Employment/Salary (full or part-time):; Eli Lilly. **X. Li:** A. Employment/Salary (full or part-time):; Eli Lilly. **G. Gilmour:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **J. Li:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **L. Rorick-Kehn:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **K. Rasmussen:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **B. Johnson:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **S.N. Mitchell:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **D. McKinzie:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **A. Nikolayev:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **V. Tolstikov:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **M. Kuo:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **P. Ornstein:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **C. Mitch:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **R. Li:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **S. Smith:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **X. Wang:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **B. Heinz:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **D. Allen:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **S. Swanson:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **J. Monn:** A. Employment/Salary (full or part-time):; Eli Lilly and Co.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.11/AA22

Topic: C.19. Drug Discovery and Development

Title: *In vivo* pharmacological properties of TAK-063, a potent and selective phosphodiesterase 10A inhibitor under investigation as a novel treatment for schizophrenia

Authors: *K. SUZUKI, A. HARADA, E. SHIRAISHI, T. TANIGUCHI, H. KIMURA;
Takeda Pharmaceut. Co. Ltd, Kanagawa, Japan

Abstract: Phosphodiesterase 10A (PDE10A) is a cAMP/cGMP phosphodiesterase highly expressed in the medium spiny neurons (MSNs) of striatum. Here, we evaluated the *in vivo* pharmacological profile of TAK-063, a potent and selective PDE10A inhibitor. TAK-063 (0.3 mg/kg, p.o.) selectively increased cAMP and cGMP contents in the rodent striatum and up-regulated phosphorylation levels of CREB and the AMPA-receptor GluR1 subunit which are key substrates of cAMP-dependent and cGMP-dependent protein kinases. TAK-063 strongly suppressed MK-801-induced hyperlocomotion and this antipsychotic-like effect by TAK-063 was maintained after repeated administration (0.5 mg/kg/day, p.o. for 15 days). Side effect profile of TAK-063 was assessed using current antipsychotics (haloperidol, olanzapine, and aripiprazole) as controls. TAK-063 did not affect plasma prolactin and glucose levels at doses up to 3 mg/kg, p.o., probably due to low expression level of PDE10A in the pituitary and selective PDE10A inhibition by TAK-063. TAK-063 at a higher dose (3 mg/kg, p.o.) exhibited weak cataleptic responses compared to haloperidol and olanzapine in rats. Evaluation of pathway-specific markers (substance P mRNA for direct pathway and enkephalin mRNA for indirect pathway)revealed that TAK-063 activated both direct and indirect pathways of MSNs. Activation of direct pathway may be responsible for this weak cataleptic response because haloperidol (D_2 antagonist)-induced cataleptic response was suppressed by activation of direct pathway by SKF82958 (D_1 agonist). These findings suggest that TAK-063 represents a promising target in the treatment of schizophrenia with potential for a benign safety and tolerability profiles.

Disclosures: **K. Suzuki:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **A. Harada:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Ltd. **E. Shiraishi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Ltd. **T. Taniguchi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Ltd. **H. Kimura:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Ltd.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.12/AA23

Topic: C.19. Drug Discovery and Development

Support: REAP Reward- VA Rehab R&D

Title: Rabies glycoprotein derived peptide shows higher capcity for delivery model-protein GFP than tetanus toxin fragment C, Tat, and Tet-1

Authors: *N. MELLO[1], P. GRAMLICH[2], M. REMMINGTON[3], P. FISMAN[1,3];
[1]Univ. of Maryland-Baltimore, Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]VA Hospital-Baltimore, Baltimore, MD

Abstract: Several peptides have been proposed as vectors to enhance binding an internalization of potentially therapeutic agents. The purpose of this study was to directly compare rabies glycoprotein derived peptide (RDP), tetanus toxin fragment C (TTC), an 11 amino acid peptide derived from the HIV Tat protein, and Tet-1 peptide derived from phage screening. The goal was to determine the capacity of each vector to deliver a test cargo protein into neuronal cells. The four vectors (RDP, TTC, Tat, and Tet-1) were linked to the N-terminus of green fluorescent protein (GFP). These four recombinant proteins were produced in bacteria and isolated using a GST-tagged system. Cell associated (bound or internalized) protein was assessed in human neural progenitor cells (hNPCs) plated in 8-well chamber slides. Cells were treated with 0.508 nM, 0.254 nm or 0.127 nM of RDP-GFP, TTC-GFP, Tat-GFP, or Tet-1-GFP. After 18 hours of exposure to the recombinant proteins, cells were washed and imaged using fluorescence microscopy and quantitative image analysis. The 3T3 fibroblast line was used as a control for non-neuronal binding (0.508 nM of each recombinant protein). Binding data was normalized on the basis of the recombinant proteins' fluorescence measured by flourimetry. Quantitative analysis of the hNPCs images showed that RDP-GFP had the most internalized fluorescent in cells, even though the basic fluorescence of the protein was the least. Tet-1-GFP showed the least internalized fluorescence, with TTC-GFP fluorescent internalization being slightly higher than Tat-GFP. RDP-GFP also showed the most fluorescent internalization in 3T3 cells while the other three recombinant proteins were internalized at similar rates. Based on this data, it was concluded that RDP has the highest capacity of the four vectors tested for delivery a test protein. RDP, TTC, and Tet-1 had been shown in previous studies to be neuron binding specific. This makes the lack of selectivity for neurons versus 3T3 cells surprising. This could have been influenced by the high concentrations of recombinant protein used in this study and high levels of non-specific endocytosis in the 3T3 cell line. In the clinical setting, the critical property of neuronal vectors is high capacity. The RDP vector is unmatched by any other vector used in this study, making it a potential candidate to deliver therapeutic agents *in vivo* in future work.

Disclosures: **N. Mello:** A. Employment/Salary (full or part-time):; University of Maryland-Baltimore. **P. Gramlich:** A. Employment/Salary (full or part-time):; VA Hospital-Baltimore, John Hopkins University. **M. Remmington:** A. Employment/Salary (full or part-time):; VA Hospital-Baltimore. **P. Fisman:** A. Employment/Salary (full or part-time):; University of Maryland Baltimore, VA Hospital-Baltimore.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

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Program#/Poster#: 713.13/AA24

Topic: C.19. Drug Discovery and Development

Support: R01 NS075156/NS/NINDS NIH HHS/United States

R01 NS075156/NS/NINDS NIH HHS/United States

Title: Long-term treatment with thymosin β4 improves functional recovery of diabetic peripheral neuropathy

Authors: *X. LU, Z. ZHANG, M. CHOP, L. JIA, A. SZALAD, L. WANG;
Neurol., Henry Ford Hosp., Detroit, MI

Abstract: Background: Peripheral neuropathy is a chronic complication of diabetes mellitus. In this study, we investigated the efficacy and safety of Tβ4 for the long-term treatment of diabetic peripheral neuropathy. Methods and Results: Male diabetic mice (C57BLKS/J-m+/+Leprdb homozygous, db/db) at age 24 weeks (n=15/group) were treated with Tβ4 (30 mg/kg, i.p. daily) or saline for 16 consecutive weeks. Treatment of diabetic mice with Tβ4 significantly ($p<0.05$) improved motor (MCV, 40±1s vs. 30±1s in control) and sensory (SCV, 43 ± 2s vs. 31 ± 2s in control) conduction velocity in the sciatic nerve and the thermal latency (radiant heat test: 9±1s vs.12±1s in control). Treatment with Tβ4 increased diabetes-induced narrowed axon area (19.1±0.48μm2 vs.17.5±0.41 μm2 in control) and myelin thickness reductions (1.85±0.04 μm vs.1.50±0.04 μm in control) and the "g" ratio increase (0.57±0.008 vs.0.61±0.008 in control; $p<0.05$) in sciatic nerve. Furthermore, Tβ4 significantly increased intra-epidermal nerve fibers density (12.6±0.5 mm2 vs.7.7±0.5 mm2 in control, $p<0.05$). Western blot analysis of the sciatic nerve revealed that Tβ4 elevated angiopoietin-1 (Ang1) levels (0.88±0.1 fold vs.0.53±0.15 fold in control). *In vitro*, compare with dorsal root ganglia (DRG) neurons derived from non-diabetic mice, DRG neurons derived from diabetic mice significantly decreased neurite outgrowth (0.7±0.05 vs. 1.0±0.01fold in the non-diabetic mice; n=4/group, $p<0.05$). Tβ4 reversed diabetes-decreased neurite outgrowth (1.6±0.1 fold) in cultured DRG neuron. Blockage of the Ang1/Tie2 signaling pathway with a naturalized antibody against Tie2 abolished Tβ4-increased neurite outgrowth (0.7±0.1 fold, $p<0.05$). Conclusion: Long term Tβ4 treatment is safe and provides therapeutic effect to improve neurological outcome in diabetic peripheral neuropathy. Tβ4 ameliorates diabetic-induced axonal degeneration, demyelination and intra-epidermal nerve fiber

impairment, which likely contribute to therapeutic effect of Tβ4 on diabetic neuropathy. The Ang1/Tie2 pathway may mediate Tβ4-induced axonal remodeling.

Disclosures: **X. Lu:** None. **Z. Zhang:** None. **M. Chop:** None. **L. Jia:** None. **A. Szalad:** None. **L. Wang:** None.

Poster

713. Drug Discovery and Delivery

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Topic: C.19. Drug Discovery and Development

Support: OTKA grant NN107234

TÉT_10-1-2011-0050 grant

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KTIA_13_NAP-A-III/1 grant

Title: Neurochemical changes in the mouse hippocampus underlying the antidepressant effect of genetic deletion of P2X7 receptors

Authors: *B. SPERLAGH, C. CSÖLLE, M. BARANYI, F. GÖLÖNCSÉR, L. OTROKOCSI, A. KITTEL;
Dept Pharmacol, Inst. of Exptl. Med., Budapest, Hungary

Abstract: Recent investigations have revealed that the genetic deletion of P2X7 receptors (P2rx7) results in an antidepressant phenotype in mice. However, the link between the deficiency of P2rx7 and changes in behavior has not yet been explored. In the present study, we studied the effect of genetic deletion of P2rx7 on neurochemical changes in the hippocampus that might underlie the antidepressant phenotype. P2X7 receptor deficient mice (P2rx7-/-) displayed decreased immobility in the tail suspension test (TST) and an attenuated anhedonia response in the sucrose preference test (SPT) following bacterial endotoxin (LPS) challenge. The attenuated anhedonia was reproduced through systemic treatments with P2rx7 antagonists. The activation of P2rx7 resulted in the concentration-dependent release of [3H]glutamate in P2rx7+/+ but not P2rx7-/- mice, and the NR2B subunit mRNA and protein was upregulated in the hippocampus of P2rx7-/- mice. The brain-derived neurotrophic factor (BDNF) expression was higher in saline

but not LPS-treated P2rx7-/- mice; the P2rx7 antagonist Brilliant blue G elevated and the P2rx7 agonist benzoylbenzoyl ATP (BzATP) reduced BDNF level. This effect was dependent on the activation of NMDA and non-NMDA receptors but not on Group I metabotropic glutamate receptors (mGluR1,5). An increased 5-bromo-2-deoxyuridine (BrdU) incorporation was also observed in the dentate gyrus derived from P2rx7-/- mice. Basal level of 5-HT was increased, whereas the 5HIAA/5-HT ratio was lower in the hippocampus of P2rx7-/- mice. The LPS-induced elevation of 5-HT level was absent in P2rx7-/- mice. In conclusion there are several potential mechanisms for the antidepressant phenotype of P2rx7 -/- mice, such as the absence of P2rx7-mediated glutamate release, elevated basal BDNF production, enhanced neurogenesis and increased 5-HT bioavailability in the hippocampus.

Disclosures: B. Sperlagh: None. **C. Csölle:** None. **M. Baranyi:** None. **F. Gölöncsér:** None. **L. Otrokocsi:** None. **A. Kittel:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.15/BB2

Topic: C.19. Drug Discovery and Development

Title: Creation of brain-penetrant iduronidase by conjugation with Angiopeps and demonstration of brain enzyme activity in a mouse model of MPSI

Authors: *J. E. LACHOWICZ, M. DEMEULE, A. REGINA, S. DAS, K. MOKTEFI, A. LAROCQUE, D. BOIVIN, S. LORD-DUFOUR, J.-P. CASTAIGNE;
Angiochem, Inc., Montreal, QC, Canada

Abstract: Mucopolysaccharidosis I (MPSI) is a rare genetic disorder resulting from mutations in the gene encoding iduronidase (IDUA), a lysosomal enzyme in the catabolic pathway of glycosaminoglycans. The most common and most severe form of the disease, Hurler's Syndrome, is associated with numerous symptoms including reduced CNS function, and if untreated, results in death at 5 to 10 years of age. Treating MPSI with enzyme replacement therapy (Aldurazyme, BioMarin) has been demonstrated to improve non-CNS aspects, such as pulmonary function and walking capacity. However, because enzymes do not cross the blood-brain barrier, these treatments have been unable to restore IDUA activity in the brain, and CNS symptoms persist. While efficient at blocking xenobiotics from entering the brain, the BBB is also adept at transporting necessary nutrients and other molecules from the circulation to the

brain. One such mechanism utilizes the LRP-1 receptor, which brings a variety of large protein ligands from the circulation to the brain by receptor-mediated transcytosis across BBB capillary endothelial cells. The peptide Angiopep-2 (An2), derived from LRP-1 ligands, has been conjugated to small molecules, peptides, and monoclonal antibodies to create brain-penetrant therapeutics. To determine whether the An2 incorporation strategy could be applied to enzymes, Angiopep-2 and derivatives were conjugated to IDUA using a variety of reaction conditions and linkers. Conjugates were analyzed by MALDI-TOF mass spectrometry to ascertain the number of Angiopep incorporated per enzyme. Systemic delivery of 125I-labeled IDUA conjugates showed a high level of variation in brain exposures for the panel of conjugates. Conjugates that entered brain parenchyma most efficiently were tested for *ex vivo* brain IDUA activity in IDUA KO mice. Mice were treated with IDUA or IDUA conjugates and brains were removed at various time points for measurement of IDUA activity using an IDUA-specific fluorogenic substrate. Significantly higher enzyme activity in brain was observed for animals treated with Angiopep-IDUA conjugates compared to native IDUA. These results suggest that CNS symptoms of MPSI may be treated using modified enzyme replacement therapy, and that the Angiopep conjugation strategy is a viable means of modifying lysosomal enzymes for CNS exposure.

Disclosures: J.E. Lachowicz: A. Employment/Salary (full or part-time):; Angiochem. **M. Demeule:** A. Employment/Salary (full or part-time):; Angiochem. **A. Regina:** A. Employment/Salary (full or part-time):; Angiochem. **S. Das:** A. Employment/Salary (full or part-time):; Angiochem. **K. Moktefi:** A. Employment/Salary (full or part-time):; Angiochem. **A. Larocque:** A. Employment/Salary (full or part-time):; Angiochem. **D. Boivin:** A. Employment/Salary (full or part-time):; Angiochem. **S. Lord-Dufour:** A. Employment/Salary (full or part-time):; Angiochem. **J. Castaigne:** A. Employment/Salary (full or part-time):; Angiochem.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.16/BB3

Topic: C.19. Drug Discovery and Development

Title: Interaction between TRPC6 channels and FKBP52 - Implications for the pathophysiology of mood disorders

Authors: L. YE[1], F. HAUSCH[2], *K. LEUNER[3];
[1]Chem. and Pharm., FAU Erlangen/Nuremberg, Erlangen, Germany; [2]Max Planck Inst. for Psychiatry, Munich, Germany; [3]Chem. and Pharm., FAU Erlangen Nuremberg, Erlangen, Germany

Abstract: The FK506 binding protein 51 (FKBP51) is a member of the immunophilin superfamily. FKBP51 acts as an Hsp90-associated co-chaperone regulating responsiveness of steroid hormone receptors. Genetic association studies revealed an association of FKBP51 with emotional processing and affective disorders such as major depression. Until now, its molecular effects were mainly considered to be mediated via steroid hormone receptors. Here, we demonstrate that part of its effects in neuronal cells might be induced by the interaction with the transient receptor potential channel TRPC6. TRPC6 channels are non-selective cation channels permeable for mono- and divalent cations such as calcium. In the CNS, TRPC6 channels are discussed to be involved in neuronal differentiation and proliferation and synaptogenesis during development but also in the adult brain. Furthermore, TRPC6 channels are the molecular target of hyperforin, the antidepressant active constituent of St. John's wort extracts which are used to treat mild to moderate depression. These findings might contribute to a better understanding of the molecular mechanism how FKBP51 and TRPC6 channels are involved in the pathogenesis of mood disorders.

Disclosures: L. Ye: None. **F. Hausch:** None. **K. Leuner:** None.

Poster

713. Drug Discovery and Delivery

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.17/BB4

Topic: C.19. Drug Discovery and Development

Title: Cerebrospinal fluid sampling after intranasal administration of kyotorphin alters brain concentrations in rats

Authors: *A. L. SVITAK, S. V. DHURIA, W. H. FREY, 2nd, L. R. HANSON;
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Abstract: Intranasal (IN) drug administration has been shown to be a rapid, non-invasive method for delivering molecules to the central nervous system (CNS) and it bypasses the blood brain barrier (BBB). Kyotorphin (D-KTP), a small morphine-like analgesic does not normally

cross the BBB, but can reach the CNS with IN delivery. This delivery can also be enhanced with the addition of a vasoconstrictor in the dose solution. Often, in order to reduce the number of animals required, studies are designed so that blood, cerebrospinal fluid (CSF), and brain tissues are obtained from the same animal after IN administration. The purpose of this study was to determine if CSF sampling had an effect on drug distribution in the CNS following IN and intravenous (IV) administration of D-KTP. Four groups of anesthetized rats were administered D-KTP either intranasally or intravenously with or without CSF sampling. CSF from the cisterna magna was sampled 25-30 minutes after the onset of drug administration. Distribution to the CNS, blood, and CSF was evaluated by gamma counting. CSF sampling had no effect on D-KTP blood concentrations following IN or IV administration. CSF sampling had a minimal effect on distribution to the brain and CSF following IV administration of D-KTP. However, following IN administration, drug distribution was dramatically affected with CSF sampling. The brain areas most affected by CSF sampling with intranasal delivery included the olfactory bulbs (4.4-fold), hypothalamus (3.6-fold), and anterior olfactory nucleus (2.6-fold). In the presence of a vasoconstrictor (1% phenylephrine, PHE), CSF sampling increased IN drug distribution up to 10-fold. The brain areas most affected included the anterior olfactory nucleus (10-fold), olfactory bulbs (8.0-fold), frontal cortex (6.4-fold), thalamus (6.2-fold), and hypothalamus (5.5-fold). These results indicate that CSF sampling via cisternal puncture in anesthetized rats following IN administration (but not IV administration) significantly alters drug distribution in the CNS. From this work, it is recommended that a separate group of animals be used for assessing drug concentrations in the CSF or alternative methods for CSF collection be explored in order to report accurate drug concentrations in CNS tissues.

Disclosures: **A.L. Svitak:** None. **S.V. Dhuria:** None. **W.H. Frey:** None. **L.R. Hanson:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.18/BB5

Topic: C.19. Drug Discovery and Development

Support: NIH Grant NS076448

Title: Rapid intranasal brain delivery of pralidoxime as a countermeasure against nerve agent poisoning

Authors: *A. NAMBOODIRI[1], J. K. S. KRISHNAN[1], A. PEETHAMBARAN[1], J. R. MOFFETT[1], W. H. FREY, II[2];
[1]USUHS, BETHESDA, MD; [2]Alzheimer's Res. Ctr. at Regions Hospital, Hlth. Partners Res. Fndn., St. Paul, MN

Abstract: Nose to brain delivery bypasses the blood brain barrier (BBB) to rapidly deliver drugs to the CNS via extracellular and paracellular routes along the olfactory and trigeminal neural pathways. The major advantage of this approach is that many charged molecules and high molecular weight compounds, which cannot penetrate the BBB, can be rapidly delivered to the CNS in therapeutic doses. The long term objective is to develop methods for intranasal delivery of neuroprotectant compounds to the brain for preventing and treating chemical threat agent mediated casualties. The immediate goal is to develop a rapid intranasal delivery method for Pralidoxime (2-PAM) and optimize effectiveness in a preclinical model system. Initial bioavailability studies involved the use of free Pralidoxime (2-PAM) and HPLC based quantitation in different brain areas. In order to improve sensitivity over the HPLC method, we then custom synthesized radiolabelled ^{3}H-2-PAM with high specific activity (20 Ci/mmole) using a published method. We administered 2-PAM (2 mg unlabeled + 130 μCi labeled in sterile saline) to the olfactory epithelium of adult rats (Sprague-Dawley, 200-250g) under anesthesia using an aerosol intranasal drug delivery device (Impel Neuropharma, WA). Animals were euthanized after different time intervals (2.5 min to 1 hour) and the brains were rapidly dissected into 9 different regions. 2-PAM was extracted using methanol and was quantified by liquid scintillation counting. We detected rapid (within 2.5 min) delivery of 2-PAM into all the brain areas with higher concentrations in the trigeminal ganglia (25-50 μM) and olfactory bulb (35-70 μM). Presently, we are testing multiple dosing schedules as well as use of dimethylsulfoxide to increase 2-PAM delivery to the brain with the goal of testing its effectiveness as a CNS-targeted countermeasure in a paraoxon model system. NIH Grant NS076448.

Disclosures: A. Namboodiri: None. **J.K.S. Krishnan:** None. **A. Peethambaran:** None. **J.R. Moffett:** None. **W.H. Frey:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.19/BB6

Topic: C.19. Drug Discovery and Development

Title: Pharmacologic properties of TAK-063, a potent and selective phosphodiesterase 10A inhibitor, as a novel therapeutic option for the treatment of schizophrenia

Authors: *H. KIMURA, K. SUZUKI, A. HARADA, E. SHIRAISHI, N. KAMIGUCHI, K. NAKASHIMA, T. TANIGUCHI;
Takeda Pharmaceut. Co. Limited, Kanagawa, Japan

Abstract: Phosphodiesterase 10A (PDE10A) is a cAMP/cGMP phosphodiesterase highly expressed in the medium spiny neurons of striatum. In this study, we assessed the pharmacological profile of TAK-063, a potent and selective PDE10A inhibitor, as a novel therapeutics for schizophrenia. TAK-063 showed potent and selective PDE10A inhibitory activity *in vitro*; IC50 for human recombinant PDE10A2 was 0.30 nmol/L and selectivity over other PDEs was more than 15,000-fold. Autoradiography study showed the selective binding of [^{3}H]TAK-063 to the native PDE10A in the rodent brain. Oral administration of TAK-063 dose-dependently increased cAMP and cGMP contents and activated its downstream signaling, such as CREB, in the rodent striatum. TAK-063 (0.3 mg/kg, p.o.) showed potent antipsychotic-like effects in rodent models of schizophrenia; TAK-063 (0.3 mg/kg, p.o.) suppressed MK-801- and methamphetamine-induced hyperlocomotion. It was reported that MP-10, a Pfizer's PDE10A inhibitor, does not improve sensorimotor gating. Interestingly, TAK-063 improved the sensorimotor gating deficits in C57BL/6J mice at doses to show antipsychotic-like effects. To know the relationship between PDE10A occupancy and pharmacological effects of TAK-063, its PDE10A occupancy in rats was measured by using T-773, a newly developed positron emission tomography tracer for PDE10A. Detailed studies to understand the difference between TAK-063 and other PDE10A inhibitors are on-going. Taken together, TAK-063, a novel, potent, and selective PDE10A inhibitor, is a promising molecule for the treatment of CNS disorders such as schizophrenia. Clinical studies have been initiated (ClinicalTrials.gov Identifiers: NCT01879722 and NCT01892189) and results will be presented in the future.

Disclosures: **H. Kimura:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **K. Suzuki:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **A. Harada:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **E. Shiraishi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **N. Kamiguchi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **K. Nakashima:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited. **T. Taniguchi:** A. Employment/Salary (full or part-time):; Takeda Pharmaceutical Company Limited.

Poster

713. Drug Discovery and Delivery

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Program#/Poster#: 713.20/BB7

Topic: C.19. Drug Discovery and Development

Support: NIMH Grant 5R44MH083417

MJFF Grant

Title: Targeting xCT (the cystine-glutamate antiporter) for the treatment of CNS disorders

Authors: *C. E. BEYER[1], M. P. NEARY[1], N. RADDATZ[2], D. C. LOBNER[2], J. R. MANTSCH[2,1], D. G. LAWTON[1], D. A. BAKER[2,1];
[1]Promentis Pharmaceuticals, Milwaukee, WI; [2]Dept. of Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Several disorders of the brain including schizophrenia, Parkinson's disease and autistic spectrum disorders (ASD) share a common neurochemical signature--dysregulation in glutamate neurotransmission and oxidative balance. The cystine-glutamate antiporter (also referred to as System xc- or xCT) has been identified as a target regulating critical sources of both extracellular glutamate and intracellular glutathione (GSH), a potent and primary antioxidant within the body. N-acetylcysteine (NAC), a drug acting on xCT, is reported to exert significant benefits in humans including but not limited to adults with schizophrenia and children with autism. These clinical proof-of-concept studies, in spite of the poor CNS bioavailability of NAC (less than 1%), support the contention that drugs designed to target xCT represent a novel therapeutic approach for a variety of disorders where glutamate dysfunction and mitochondrial / oxidative stress are key neurochemical underpinnings. Promentis Pharmaceuticals (www.promentispharma.com) has designed, synthesized and characterized a novel library of proprietary molecules designed to target xCT. In our pursuit of discovering novel xCT ligands, we generated PRO-4051 and related derivatives that drive xCT *in vitro*, are selective over a broad panel of off-target receptors/transporters and possess superior brain penetration compared to NAC following oral (p.o.) administration. Further, PRO-4051 (3-30 mg/kg, p.o.) produces a significant ($p<0.05$) reversal of deficits induced by MK-801 (0.1 mg/kg) in pre-pulse inhibition (PPI) and increases the time spent in the open arm of the elevated plus maze (EPM)--responses suggestive of antipsychotic- and anxiolytic-like activity, respectively. With continued pre-IND success, and a keen eye focus on leveraging the purported efficacy and safety of NAC in humans, Promentis anticipates advancing a novel set of small molecules closer to the millions of patients and their families living with certain CNS disorders.

Disclosures: C.E. Beyer: A. Employment/Salary (full or part-time):; Promentis Pharmaceuticals. 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.; NIMH Grants. **M.P. Neary:** A. Employment/Salary (full or part-time):; Promentis. **N. Raddatz:** A. Employment/Salary (full or part-time):; Marquette University. **D.C. Lobner:** A. Employment/Salary (full or part-time):; Marquette. **J.R. Mantsch:** A. Employment/Salary (full or part-time):; Marquette. **D.G. Lawton:** A. Employment/Salary (full or part-time):; Promentis. **D.A. Baker:** A. Employment/Salary (full or part-time):; Marquette.

Poster

713. Drug Discovery and Delivery

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Program#/Poster#: 713.21/BB8

Topic: C.19. Drug Discovery and Development

Support: NINDS/NIH grant R21-NS066984

Title: Brain distribution of gd-nanoemulsion upon intranasal delivery using 3d atlas based MRI study

Authors: *P. P. KULKARNI[1], S. YADAV[2], M. AMIJI[2], C. F. FERRIS[1];
[1]Psychology, [2]Pharmaceut. Sci., Northeastern Univ., BOSTON, MA

Abstract: Several bio-therapeutics including monoclonal antibodies, peptides, proteins etc. have shown promise in treatment of various brain disorders however, their systemic delivery across the BBB has been a major limitation. We examined uptake of Gadolinium-nanoemulsion (NE-Gd3+), in rats brain via intranasal delivery. The Gadolinium (Gd3+) ion shortens the T1 relaxation time. We examined changes in T1 relaxation value as measure of NE-Gd3+ uptake. NE-Gd3+ were prepared by a high energy ultra-sonication method using biocompatible and omega-3 rich fatty acids. These systems was further characterized for particle size, zeta potential and in-vitro relaxation time. *In vivo* imaging was conducted using a Bruker Biospec 7.0T/20-cm USR horizontal magnet. RF signal was sent and received with the quad coil (Animal Imaging Research) electronics built into the animal head restrainer. T1 relaxation was measured using RAREvtr pulse sequence with 5 TR values. 22 axial slices of 1 mm thickness with 128 ×128 in plane resolution (3 cm FOV) were collected. T1 measurements were computed using Paravision 5.1 software by fitting absolute signal at particular TR. The T1 value scans were then registered to 3D brain Atlas and segmented into more than 150 regions. T1 value for each region was computed by calculating average T1 value for all the voxels in that ROI. The study was

conducted in two groups (n=6), T1 measurements were done before intranasal delivery and three time points after delivery. Uptake in the brain regions were computed by comparing changes in T1 value at particular time point with control group. Out of 150 segmented regions 22 specific regions of the brain show statistically higher changes demonstrating specific uptake into those regions. Magnevist delivered as a solution failed to show any differences when compared to control group or to NE-Gd3+ groups. This present study utilizing MRI as a tool demonstrates that NE-Gd3+ can bypass the BBB to reach multiple sites within the brain approximately at 30min time point after the intranasal administration. We believe this approach opens a window of opportunity to target variety of diseases within the nervous system, where exposure of drugs is a major limitation.

Disclosures: **P.P. Kulkarni:** A. Employment/Salary (full or part-time):; Northeastern University. **S. Yadav:** None. **M. Amiji:** None. **C.F. Ferris:** None.

Poster

713. Drug Discovery and Delivery

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Program#/Poster#: 713.22/BB9

Topic: C.19. Drug Discovery and Development

Support: NSFC Grant 90913019

Title: Potassium channel TREK-1 might be a potential target for treatment of depression

Authors: *X. WANG;
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Abstract: The pathogenesis of Major depressive disorder (MDD) is still unclear. Recent reports indicated that potassium channels especially two pore domain potassium channels might participate in the pathogenesis of MDD through regulating the excitability of neurons. The purpose of the present study is to demonstrate the relationship between TREK-1 channels and MDD as well as the possibility of TREK-1 as a target for treatment of MDD. The force swim test (FST), tail suspension test (TST) and chronic mild stress (CMS) depression animal models were used in our studies. The stress induced significant decrease of 5-HT and NE level in the hippocampus and prefrontal cortex were found. The expressions of potassium channel subunits in CMS rats were observed. Our results showed that the expression of TREK-1 was significantly increased in the prefrontal cortex in CMS animal model. The enhanced TREK-1 was

significantly down regulated in the prefrontal cortex after 21-days treatment with fluoxetine. In TREK-1 highly expressed cell line (TREK-1/Hek293) fluoxetine inhibited the current of TREK-1 concentration dependently measured by patch clamping techniques. L-NBP, the parent drug of potassium 2-(1-hydroxypentyl)-benzotate, *dl*-PHPB, an anti-ischemic stroke new drug candidate (a novel compound designed and synthesized by Institute of Materia Medica, Chinese Academy of Medical Sciences) showed a highly potent in blocking TREK-1 channels with a IC50 of 2 μM. DL-PHPB also showed significant effect on CMA depression model and induced down-regulation of TREK-1 channels in cortex after three weeks treatment like fluoxetine. The effects of dl-PHPB were not associated to the changes of 5-HT and NE in the brain. Because, it's anti-depress effect was not completely abolished by pre-treating the mice with pCPA (an inhibitor of 5-HT synthesis). It indicates that the anti-depress action of PHPB may not mainly dependent on the serotonergic system. Conclusion, our results demonstrated that potassium channel TREK-1 might involved in the pathology of depression and it might be the new target for treatment of the disease.

Disclosures: X. Wang: None.

Poster

713. Drug Discovery and Delivery

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Topic: C.19. Drug Discovery and Development

Support: NIH R01NS063360

Fulbright-COLCIENCIAS-DNP

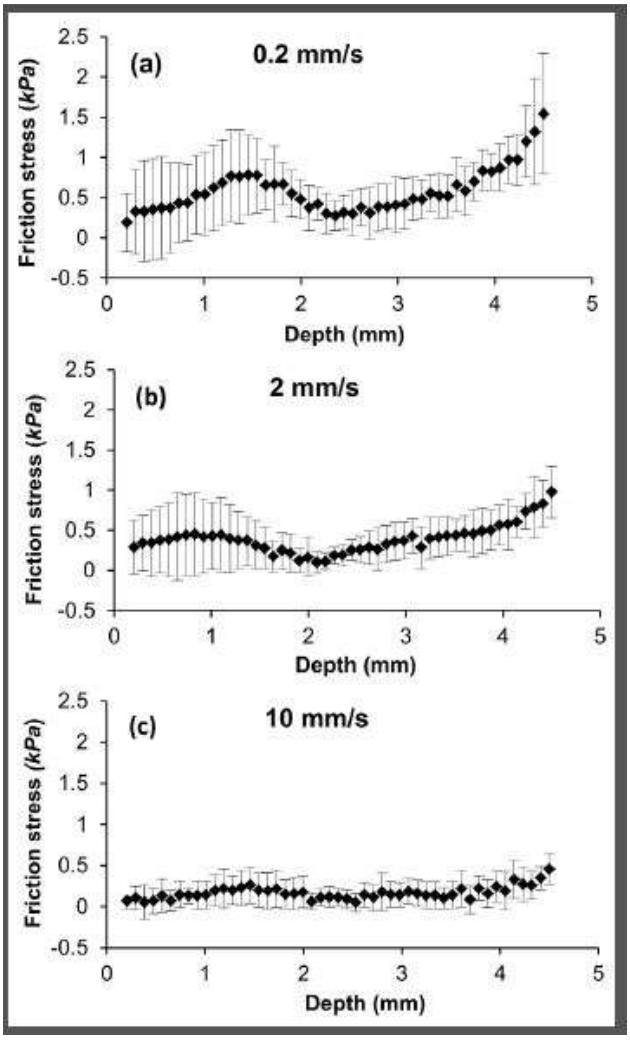
Universidad del Valle, Cali, Colombia

Title: In-vivo evaluation of tissue damage during insertion of needles at varying insertion speed into the brain

Authors: *M. SARNTINORANONT[1], F. CASANOVA[1], P. F. CARNEY[2];
[1]Mechanical & Aerospace Engin., [2]Dept. of Pediatrics, Univ. of Florida, Gainesville, FL

Abstract: Convection-enhanced delivery (CED) is a technique that infuses drugs directly into central nervous system tissue. Needle insertion is required for CED and results in direct tissue damage, which can promote flow back along the needle track and improper targeting. Damage is

related to needle-tissue interactions, and the goal of this study was to measure changes in the mechanics of insertion for varying speeds: 0.2, 2, and 10 mm/s. Needle insertion forces and surface displacement (surface dimpling) during insertion into the caudate putamen (CPu) were measured in rat brain *in vivo*. Average insertion force for 32 g needles increased with increasing insertion speed (0.92 ± 0.60 to 1.77 ± 0.92 mN). Surface dimpling before puncture also increased with insertion speed (0.65 ± 0.17 to 0.90 ± 0.22 mm). Needle retraction force was used to determine *in vivo* friction stress (product of tissue stress and friction coefficient) along the needle track. Average friction stress along the needle-tissue interface decreased with insertion speed (0.58 ± 0.27 to 0.16 ± 0.08 kPa). These trends indicate tissue damage is increased with increasing needle insertion speed. Damage and friction stress also varied between brain regions: cortex (0.227 ± 0.27 kPa), external capsule (0.222 ± 0.19 kPa), and CPu (0.383 ± 0.30 kPa). Real-time measures of needle force were found to be clearly sensitive to insertion rate and are potential predictors of the extent of tissue damage. Friction stress also provides a measure of spatially varying tissue damage along the tissue track. Significant damage (reductions in friction stress) within white matter regions has important implications for understanding why these regions are susceptible to CED backflow. In addition, this study highlights the importance of using lower insertion speeds when introducing infusion needles in order to minimize tissue damage, decrease CED backflow and improve targeting. Fig. 1. Friction stress vs. needle depth for varying insertion speeds in brain tissue.



Disclosures: **M. Sarntinoranont:** None. **F. Casanova:** None. **P.F. Carney:** None.

Poster

713. Drug Discovery and Delivery

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NSF (DMR-0526686)

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NKU Research Foundation

Title: Gene expression and cytotoxicity in an *in vitro* model of the blood-brain barrier following poly(butylcyanoacrylate) nanoparticle exposure

Authors: A. HALL[1], R. HEMMER[1], R. SPAULDING[1], H. N. WETZEL[1], B. A. SABEL[3], P. HENRICH-NOACK[3], S. PIXLEY[4], T. HOPKINS[4], R. BOYCE[1], H. A. BULLEN[2], P. SCHULTHEIS[1], *K. L. HAIK[1];
[1]Dept Biol. Sci., [2]Dept Chem., Northern Kentucky Univ., NEWPORT, KY; [3]Inst. of Med. Psychology, Otto-von-Guericke Univ. Sch. of Med., Magdeburg, Germany; [4]Vontz Ctr. for Mol. Studies, Univ. of Cincinnati Med. Ctr., Cincinnati, OH

Abstract: Poly(butylcyanoacrylate) (PBCA) nanoparticles (NP) coated with polysorbate 80 (PBCA-PS80) have been shown to deliver several drugs to the brain that are unable to cross the blood-brain barrier (BBB) in free form. However, a detailed characterization of PBCA NP toxicity on BBB cells has not been published. This study examines potential toxicity of BBB cells after exposure to PBCA NP. A cell culture model of the BBB consisting of primary rat brain astrocytes and endothelial cells was used to evaluate cytotoxicity and to identify differences in apoptosis-related gene expression following exposure to uncoated PBCA NP, PBCA-PS80 or PBCA NP loaded with doxorubicin and coated with PS80 (PBCA-PS80-Dox). Cocultures were treated with six concentrations (0.49 to 500 µg/ml) of each NP. Resazurin cytotoxicity assays showed that uncoated PBCA NP and PBCA-PS80 NP exhibit significant toxicity at the highest concentration, while PBCA-PS80-Dox NP exhibited significant toxicity at lower concentrations. For the gene expression assays, cells were treated with PBCA NP at 7.8 µg/ml and 31.25 µg/ml and RNA was analyzed for expression of 84 apoptosis-related genes. The results show that cocultures exposed to PBCA-PS80-Dox NP exhibit alterations in 18 apoptosis-related genes compared to untreated cells. Many of the genes identified participate in the tumor necrosis factor receptor-1 (TNFR1) apoptosis pathway, indicating that PBCA-PS80-Dox NP toxicity is mediated through this pathway. No significant changes in the expression of apoptosis-related genes were detected in cocultures exposed to uncoated PBCA NP or PBCA-PS80 NP. Nano-scale hyperspectral imaging was used to confirm the presence of PBCA-PS80-Dox NP within astrocytes, demonstrating the capability of NP to cross the BBB. These results suggest that while PBCA-PS80 NP do not induce toxicity or apoptosis in this coculture system, they can effectively deliver Dox to target cells.

Disclosures: **A. Hall:** None. **K.L. Haik:** None. **R. Hemmer:** None. **R. Boyce:** None. **P. Schultheis:** None. **H.A. Bullen:** None. **B.A. Sabel:** None. **P. Henrich-Noack:** None. **S. Pixley:** None. **T. Hopkins:** None. **H.N. Wetzel:** None. **R. Spaulding:** None.

Poster

713. Drug Discovery and Delivery

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.25/BB12

Topic: C.19. Drug Discovery and Development

Support: R44MH099726 NIH

Title: Novel Pharmacomap technology to improve predictability of human outcomes based on animal preclinical studies

Authors: L. KADIRI[1], M. CASTELLI[2], K. U. VENKATARAJU[2], *P. OSTEN[3];
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Abstract: Certerra's drug-screening methodology is based on visualizing drug-evoked neural activation in the whole mouse brain at cellular resolution, using an automated microscopy, called serial two-photon (STP) tomography (Ragan et al., 2012), and a custom-built pipeline of image processing and computational methods. STP tomography generates unbiased brain-wide maps of drug-evoked c-fos activation at cellular resolution (PharmacomapTM). Here we used PharmacomapTM technology to generate database of 20 mental health medications and showed that these drugs are distinguishable from each other based on their unique PharmacomapTM "fingerprints". We performed principal component analysis which identifies the dimensions within the multidimensional dataset over which the data varies most strongly. We report clear evidence of detectable differences between all drug groups, which provides a strong support for our hypothesis that PharmacomapTM patterns can differentiate between a broad range of psychiatric medications. We also show that average PharmacomapTM datasets generated from 5 animals (per treatment group) can be attributed to a particular drug correctly with a high likelihood of 99.2%. Finally, we used the method of a linear discriminator to correlate the PharmacomapTM brain activation/inhibition patterns and the drugs' clinical indications curated from medical literature; the predictor performed significantly better, by approximately 13%, than a random predictor (we expect the predictor performance significantly improve as more drugs are added to the database). Taken together, our results suggest that the PharmacomapTM technology may provide much needed improvement in predictability of human outcomes based on animal preclinical studies.

Disclosures: L. Kadiri: None. **P. Osten:** None. **M. Castelli:** None. **K.U. Venkataraju:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

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Program#/Poster#: 713.26/BB13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: USPHS Grant P50 MH103222

Title: Oral tryptophan administration in healthy humans: effects on blood kynurenines and verbal learning

Authors: *M. A. THOMAS, L. M. ROWLAND, H. H. HOLCOMB, H. J. WEHRING, B. A. FISHER, J. WEST, R. SCHWARCZ;
Maryland Psychiatric Res. Ctr. Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Elevated levels of kynurenic acid (KYNA), a metabolite of the kynurenine pathway (KP) of tryptophan (TRYP) degradation, are found in the brain of individuals with schizophrenia (SZ) and have been suggested to be causally involved in the cognitive deficits seen in the disease (cf. Schwarcz et al., Nat. Rev. Neurosci., 13: 465-477, 2012, for review). Modest increases in brain KYNA levels cause a number of cognitive impairments reminiscent of SZ in experimental animals but this effect has not been directly investigated in humans. To address this issue, we performed a preliminary double-blinded, placebo-controlled human study focused on the relationship between the KP and cognitive function. Healthy participants (7 males, 3 females; mean age: 40.3 yrs) fasted overnight before rapidly (1 min) consuming a slurry containing 6 g of TRYP (Ajinomoto) or placebo. Serum kynurenine and KYNA levels, mood, psychiatric symptoms, subjective sleepiness, neuropsychological measures of verbal and visual memory, and working memory were assessed. Thirty minutes following the ingestion of TRYP, a steady rise occurred in blood levels of KYNA and its immediate bioprecursor kynurenine. ~10 (kynurenine) and ~100 (KYNA) fold basal levels were reached by the time the behavioral experiments concluded after 4 hrs. No changes in either metabolite occurred after placebo. Subjects reported no significant side effects with TRYP compared to placebo ingestion and showed no significant change in mood, subjective sleepiness, or psychiatric symptoms. Cognitively, TRYP ingestion was associated with a significant decline in verbal learning, as assessed with the Hopkins Verbal Learning Test (HVLT) ($t(9) = 2.23$, $p = 0.05$), and a modest decline in visual spatial learning, assessed with the Brief Visual-Spatial Memory Test (BVMT) ($t(9) = 1.67$, $p = 0.13$). TRYP did not cause any changes in verbal or visual spatial delayed recall. Finally, there were no statistically significant differences in visual discrimination accuracy ($p=0.93$) or reaction time

(p=0.58) between TRYP and placebo. The TRYP challenge methodology described here will be useful for assessing the effects of KYNA synthesis inhibitors in healthy and diseased individuals.

Disclosures: **M.A. Thomas:** None. **L.M. Rowland:** None. **H.H. Holcomb:** None. **H.J. Wehring:** None. **B.A. Fisher:** None. **J. West:** None. **R. Schwarcz:** None.

Poster

713. Drug Discovery and Delivery

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Support: University of Eastern Finland Innovative Research Initiatives fund

Academy of Finland: Project 134893

Doctoral Programme of Molecular Medicine, University of Eastern Finland

Title: NMDA receptor signaling - approaches for target identification and development of inhibitory strategies

Authors: ***L.-L. LI**[1], T. LAITINEN[2], A. POSO[2], M. J. COURTNEY[1];
[1]Dept. of Neurobio., Mol. Signalling lab, A.I. Virtanen Inst., Kuopio, Finland; [2]Computat. drug design, Sch. of Pharmacy, Univ. of Eastern Finland, Kuopio, Finland

Abstract: N-Methyl-D-Aspartate receptor (NR) signaling has great yet largely unrealized translational potential. The pathways mediated by the ternary complex composed of NR, PSD95 and neuronal nitric oxide synthase (nNOS) are known to play important roles in neuropsychiatric disorders. However, NR antagonists have failed in clinical trials because of severe side effects and nNOS inhibitors have yet to emerge in such trials despite decades of drug development. In recent years, the targeting of protein-protein interactions in this pathway has become an attractive approach. For example NA-1/NR2B9C, a peptide mimicking the NR ligand for the PDZ domains of nNOS, was the first successful neuroprotectant in clinical trials for stroke. We identified the nitric oxide synthase 1 adaptor protein (NOS1AP) downstream of this ternary complex as a critical signaling mediator in excitotoxicity, which is thought to be one of the mechanisms responsible for mental disorders. Mounting evidence from an accumulation of genetic studies has linked both nNOS and NOS1AP genes to schizophrenia, PTSD, autism and depression. Thus PSD95-nNOS interaction has recently been suggested as a novel drug target to

treat depression, and nNOS-NOS1AP interaction as a target in schizophrenia. While investigating nNOS-NOS1AP interaction in excitotoxicity we noted a discrepancy between the tight *in vitro* interaction in cell-free systems and relatively weak *ex vivo* interaction using cell lysates. It is notable that NOS1AP, the best characterized ligand for the PDZ domain of nNOS, bears a classII PDZ-motif at its C-terminus whereas nNOS-PDZ domain shows classIII selectivity. To address these anomalies, we applied a combination of mutational mapping, protein-protein interaction methods, molecular modelling and in silico screening and cell-based interaction screens to help identify determinants of the nNOS-NOS1AP interaction. The data obtained reveals an unexpectedly complex mechanism of interaction between nNOS and NOS1AP. We conclude that the interaction of nNOS-NOS1AP does not conform to a canonical PDZ domain-ligand interaction, and this finding may provide increased opportunities for development of inhibitory strategies.

Disclosures: L. Li: None. **T. Laitinen:** None. **A. Poso:** None. **M.J. Courtney:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.28/BB15

Topic: C.19. Drug Discovery and Development

Support: French Ministry of Research

Title: *In vivo* characterization of a novel highly potent GPR88 agonist

Authors: *B. L. KIEFFER[1], J. BECKER[2], D. FILIOL[2], C. MORICE[3], S. ROPP[3], S. MAYER[4], C. CATHARY[3], S. SCHANN[4];
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Abstract: GPR88 is an orphan member of the G-protein coupled receptor (GPCR) superfamily. This receptor is enriched in the striatum and was therefore proposed as a potential therapeutic target for motor dysfunctions such as Parkinson's disease or Huntington's disease (Massart et al 2009). Its roles in psychiatric disorders have also been studied and GPR88 KO mice were shown to display psychosis-like behavior (Logue et al 2009) and hyperactivity (Quintana et al 2012). Using multiple screening approaches, new GPR88 agonists were discovered. These molecules

are characterized using a native system that involves a GTPγS assay based on striatal membranes from wild type and GPR88 KO mice. Hit-to-lead efforts enable the discovery of potent agonist showing EC50 < 50nM. One of these agonists was used *in vivo* and the results of its characterization will be presented in the present poster.

Disclosures: **B.L. Kieffer:** None. **J. Becker:** None. **D. Filiol:** None. **C. Morice:** A. Employment/Salary (full or part-time):; Prestwick Chemical. **S. Ropp:** A. Employment/Salary (full or part-time):; Prestwick Chemical. **S. Mayer:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **C. Cathary:** A. Employment/Salary (full or part-time):; Prestwick Chemical. **S. Schann:** A. Employment/Salary (full or part-time):; Domain Therapeutics.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 713.29/BB16

Topic: C.19. Drug Discovery and Development

Support: NIH GRANT EB009041

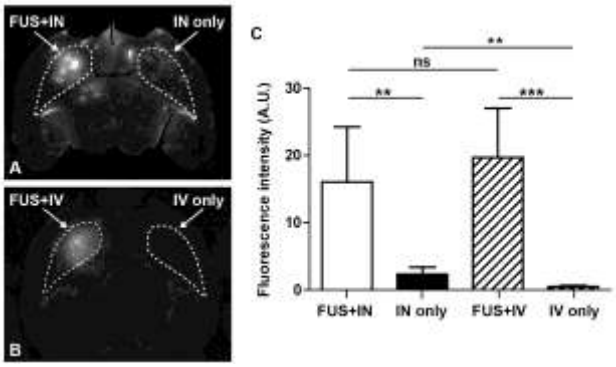
NIH GRANT AG038961

Title: A new brain drug delivery strategy: Focused ultrasound-enhanced intranasal drug delivery

Authors: ***H. CHEN**, C. C. CHEN, S.-Y. WU, T. SUN, C. ACOSTA, E. E. KONOFAGOU, E. E. KONOFAGOU;
Columbia Univ., New York, NY

Abstract: The blood-brain barrier (BBB) represents one of the strictest barriers for drug delivery. Intranasal delivery (IN) is a novel and non-invasive approach that allows direct drug delivery to the brain through the nose-brain pathway, bypassing the BBB. However, drugs are delivered to the brain in a global manner, not target-specific. Focused ultrasound (FUS) technique can increase the permeability of the BBB at the targeted location, allowing targeted delivery of intravenously (IV) injected drugs. However, IV is associated with systemic circulatory exposure. The present study revealed for the first time that the synergistic brain drug delivery effects of the FUS technique and IN administration (FUS+IN) not only circumvented the BBB but also enhanced targeted drug delivery. Fluorescently-labeled dextran was used as the model drug. It was administered via IN route before FUS technique was applied to the left

caudate putamen of mice. The contralateral side was used as control for IN only. The acoustic pressure was at diagnostic ultrasound level. For comparison, IV injection of the same amount of dextrans during FUS sonication was performed in another group of mice. The dextran delivery outcome was evaluated based on fluorescence images of brain slices. Our results confirmed that drugs can be delivered to the brain directly through the nose by IN administration only (Fig.A). FUS+IN significantly increased the amount of dextrans delivered to the targeted caudate putamen by 8-fold compared with IN only (Figs.A and C), demonstrating that FUS+IN can enhance the bioavailability and localization of drugs delivered through IN. It was also found that the amount of dextran delivered by FUS+IN reached a level comparable to that of FUS+IV (Figs.B and C), suggesting that FUS+IN constitutes an alternative strategy for brain drug delivery. For the first time the feasibility of targeted intranasal drug delivery to the brain using FUS+IN was demonstrated. Our results highlighted the possibility to develop this technology for an even wider range of therapeutic agents with minimal systemic exposure.



Disclosures: **H. Chen:** None. **C.C. Chen:** None. **S. Wu:** None. **T. Sun:** None. **C. Acosta:** None. **E.E. Konofagou:** None. **E.E. Konofagou:** None.

Poster

713. Drug Discovery and Delivery

Location: Halls A-C

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Program#/Poster#: 713.30/BB17

Topic: C.19. Drug Discovery and Development

Support: Mentored Training Fellowships - IRSF

Simons Foundation

Nancy Lurie Marks Family Foundation

NIMH Silvio Conte Center 1P50MH094271

Title: Otx2-based rescue of parvalbumin circuit connectivity in autism spectrum disorders

Authors: *A. PATRIZI[1], J. J. LEBLANC[1], S. B. MIERAU[1], M. FAGIOLINI[1], T. K. HENSCH[1,2];
[1]Neurol., Boston Children's Hosp. Harvard Med. Sch., Boston, MA; [2]Dept. of Mol. & Cell. Biol., Ctr. for Brain Science, Harvard Univ., Cambridge, MA

Abstract: The Otx2 homeoprotein has recently been identified as a potent regulator of parvalbumin (PV)-cell state and critical period brain plasticity (Sugiyama et al., 2008). By binding to the peri-neuronal net (PNN) surrounding PV-cells, Otx2 gradually accumulates within these inhibitory neurons and promotes their functional maturation. Removal of PNNs, or directly competing with Otx2 recognition of their chondroitin-sulfate proteoglycans by a small molecule inhibitor (RK peptide), resets mature PV-cells to a juvenile state and re-opens critical period plasticity in adulthood (Beurdeley et al., 2012). Here, we leverage these insights as a therapeutic tool for neurodevelopmental disorders associated with a hyper-connectivity of PV circuitry. Mice carrying a point mutation in the neuroligin-3 gene (R451C) found in some autistic patients display an enhanced inhibitory synaptic transmission (Tabuchi et al., 2007), including PV-connectivity in V1. Notably, these animals show a pronounced inhibitory phase of the visual-evoked potential (VEP) and a hyper-acuity. RK peptide infusion for one week downregulated PV expression and corrected functional VEP morphology / acuity phenotypes in adulthood. The Rett syndrome mouse model of Mecp2-deficiency also exhibits premature, enduring hyper-connectivity of PV-cells that precedes functional regression of vision beyond P30. Early sensory deprivation is sufficient to rescue this PV-hyper-maturation in Mecp2 KO mice (Durand et al., 2012). We injected RK peptide directly into V1 and observed a rapid reduction of Otx2 and PV expression followed by a gradual normalization of peri-somatic axon bouton number and loss of PNNs over a 5 to 15-day timecourse. These anatomical changes were paralleled by a reduction of intrinsic fast-spiking properties. As Otx2 synthesis at its main source (retina, choroid plexus) was unaffected by sensory experience, our results further indicate that the prevention of visual regression and PV-circuit hyper-connectivity by dark-rearing may reflect in part a reduced activity-dependent transfer of Otx2 in Mecp2 KO mice. Conversely, these results suggest acute down-regulation of Otx2 production, perhaps in the choroid plexus, as a potential therapeutic route for correcting PV-circuit connectopathies.

Disclosures: A. Patrizi: None. **J.J. LeBlanc:** None. **S.B. Mierau:** None. **M. Fagiolini:** None. **T.K. Hensch:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.01/BB18

Topic: C.19. Drug Discovery and Development

Support: Cinvestav-IPN

ESM-IPN

Title: Antinociceptive synergistic interaction between tramadol with palmitoylethanolamide on the formalin test in mice

Authors: *P. M. RAMÍREZ[1,2], M. DÉCIGA-CAMPOS[3], F. J. LÓPEZ-MUÑOZ[4]; [1]CINVESTAV, MEXICO CITY, Mexico; [2]Sección de Estudios de Posgrado e Investigación ESM, [3]Inst. Politécnico Nacional, Mexico City, Mexico; [4]Farmacobiología, Cinvestav-Sur, Mexico City, Mexico

Abstract: The purpose of this study was to evaluate the possible synergistic antinociceptive interaction between tramadol, a synthetic opioid agonist, with palmitoylethanolamide (PEA), an inhibitor of fatty acid amide hydrolase (FAAH). PEA is an endogenous lipid neuromodulator that mediates a broad spectrum of pharmacological effects by activation of peroxisome proliferator-activated receptor alpha (PPAR-alpha). There is no pharmacological evidence about the interaction between both drugs. Local injection of tramadol (1-56.2 μg/paw) and PEA (0.1-56.2 μg/paw) produced dose dependent antinociceptive effect on the formalin test in mice. An isobolographic analysis was employed to characterize the synergism produced by 1:1 fixed ratio combination of equi-effective doses of tramadol with PEA. The fifty effective concentration (EC50) of tramadol (EC50= 26.01±5.96 μg/paw) and PEA (EC50= 23.70±10.6 μg/paw) was used for the isobolographic analysis. The theoretical EC50 value for the combination estimated from the isobologram was 24.85, this value was significantly higher than the experimental EC50 value 9.5 ± 4.65 μg/paw. Results indicate that local injection of tramadol and PEA can interact synergistically to reduce inflammatory pain in mice and suggest the use of this combination to relieve pain in humans. The exact comprehension of the mechanism involved need further investigation

Disclosures: **P.M. Ramírez:** None. **F.J. López-Muñoz:** None. **M. Déciga-Campos:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.02/BB19

Topic: C.19. Drug Discovery and Development

Support: SEPI-ESP

Title: Synergistic interaction between morphine and tizanidine in rat

Authors: *M. DECIGA-CAMPOS, K. BELTRÁN-VILLALOBOS;
Escuela Superior De Medicina IPN, D.F. Mexico, Mexico

Abstract: This study was designed to evaluate the antinociceptive effect generated by tizanidine, an alfa2-adrenergic agonist, and morphine coadministration. The effects of individual and tizanidine-morphine combinations were assayed using a preclinical model of inflammatory pain. Nociception was induced in male rat by 2% formalin injection and reduction of number of flinches was considered as antinociceptive effect. Local injection of tizanidine (0.01-10 ug/paw) and morphine (0.01-30 ug/paw) generated concentration dependent antinociceptive effect. When both drugs were combined, the antinociceptive effect obtained were significantly greater compared with either drug alone or saline. An isobolographic analysis was employed to characterize the synergism produced by 1:1 fixed ratio combination of equi-effective doses of tizanidine with morphine. The fifty effective concentration (EC50) of tizanidine (EC50= 0.12±0.02 ug/paw) and morphine (EC50= 15.57±2.26 ug/paw) was used for the isobolographic analysis. The theoretical EC50 value for the combination estimated from the isobologram was 7.84, this value was significantly higher than the experimental EC50 value 0.97 ± 0.28 ug/paw. Results indicate that local injection of tizanidine and morphine can interact synergistically to reduce inflammatory pain in rat and suggest the use of this combination to relieve pain in humans. The exact comprehension of the mechanism involved need further investigation.

Disclosures: **M. Deciga-Campos:** None. **K. Beltrán-Villalobos:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.03/BB20

Topic: C.19. Drug Discovery and Development

Title: Phase I studies to evaluate safety, tolerability and pharmacokinetics of novel translocator protein antagonist ONO-2952 in healthy subjects

Authors: A. T. WOOD[1], *K. MITSUI[2], F. SUTO[1], M. KOBAYASHI[2], M. KOMENO[2], S. KATSUMATA[2];
[1]Drug Develop., ONO Pharma USA, Inc., Lawrenceville, NJ; [2]Minase Res. Institute, ONO Pharmaceut. Co., Ltd., Osaka, Japan

Abstract: The neurosteroids are known to act as allosteric modulators of excitatory and/or inhibitory neurotransmission and their synthesis is regulated by the translocator protein 18kDa (TSPO). We have demonstrated that ONO-2952, a novel and selective TSPO antagonist, inhibits stress-induced defecation and visceral hyperalgesia in rat stress models, suggesting a therapeutic potential in the treatment of stress-related disorders such as irritable bowel syndrome (IBS). The safety, tolerability, and pharmacokinetics (PK) of ONO-2952 were evaluated following single or multiple oral doses in healthy subjects. Forty-eight healthy subjects were evaluated in 6 single-dose cohorts (3 to 400 mg), including a food-effect evaluation for 2 doses (10 and 200 mg) and thirty-six healthy subjects were evaluated in 3 cohorts (30, 60, 100 mg QD) for 21 days. ONO-2952 was well tolerated and no death or serious adverse events were seen during the studies. Overall 11 adverse events (AEs) were reported in single-dose cohorts and 27 AEs were reported in multiple-dose cohorts. Except for one subject in the 60 mg multiple dose cohort who experienced constipation and was lost to follow-up, all AEs resolved by the end of the study period. There was no evidence of a dose relationship in regard to the AEs. Following a single oral dose under fasted condition the AUClast increased dose proportionally from 3 to 30 mg, but less than dose proportionally above 30 mg and the Cmax increased less than dose proportionally over the dose range tested. Dosing under fed conditions resulted in increases in both AUClast and Cmax. After multiple doses AUC24h and Cmax increased dose proportionally from 30 to 100 mg. The PK of ONO-2952 reached steady state by 10 days for each dose. The geometric mean accumulation of AUC24h ranged from 2.23 to 2.73, and that of Cmax ranged from 1.56 to 1.85. These results support the rationale for additional clinical trials to evaluate the efficacy of ONO-2952 in IBS patients which are currently underway.

Disclosures: **A.T. Wood:** A. Employment/Salary (full or part-time):; ONO Pharma USA, Inc. **K. Mitsui:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd. **F. Suto:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd. **M. Kobayashi:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd. **M. Komeno:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd. **S. Katsumata:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd..

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.04/BB21

Topic: C.19. Drug Discovery and Development

Title: A phase I PET study to evaluate brain translocator protein occupancy by ONO-2952 in healthy adult subjects using ^{11}C-PBR28

Authors: G. W. FRANKLE[1], R. NARENDRAN[1], S. N. MASON[1], A. T. WOOD[2], F. SUTO[2], K. MITSUI[3], M. KOBAYASHI[3], T. OHNO[3], A. YAMAUCHI[3], M. KOMENO[3], *S. KATSUMATA[4];
[1]Univ. of Pittsburgh Med. Ctr. Presbyterian Hosp., Pittsburgh, PA; [2]Drug Develop., ONO Pharma USA, Inc., Lawrenceville, NJ; [4]Discovery Res. Labs. I, [3]ONO Pharmmaceutical Co., Ltd., Osaka, Japan

Abstract: Translocator protein 18kDa (TSPO) has been proposed to have many physiological functions including regulation of neurosteroid biosynthesis. Pharmacological studies using rats have shown that ONO-2952, a novel TSPO antagonist, inhibits stress-induced brain neurosteroid production and noradrenaline release, suggesting a therapeutic potential of this compound for the treatment of stress-related disorders, such as irritable bowel syndrome. Moreover, several studies have revealed that ONO-2952 produces anti-stress effects in rat models and these beneficial effects correlate with the brain TSPO occupancy. This study was designed to evaluate the correlation between plasma concentration and brain TSPO occupancy by ONO-2952 in healthy subjects using ^{11}C-PBR28, a TSPO specific PET ligand, prior to evaluating disease efficacy in phase II trials. Sixteen healthy subjects were scanned twice with ^{11}C-PBR28 PET. A baseline, off medication, scan was performed first, with a second scan performed 24 hours after a single oral dose of ONO-2952 (either 200, 60, 20, or 6 mg). ^{11}C-PBR28 distribution volumes (VT) were determined by kinetic analysis using a two tissue compartment model with arterial input for 15 regions of interest (ROIs). Whole brain TSPO occupancy by ONO 2952 was obtained as the slope of the regression line for VTbase - VTdrug plotted against VTbase (Lassen plot). Regional occupancy was calculated as the change in binding potential relative to non-specific binding from each individual's baseline, with nonspecific binding derived from the Lassen plot as Vnd = Intercept/-slope. TSPO occupancy in each ROI and whole brain generally increased with plasma concentration and then began to level off at maximal binding level. ONO-2952 at 200 mg demonstrated a mean TSPO occupancy as high as 96% with close to 60% of a mean TSPO

occupancy at 6 mg. Overall, there were no inter-regional differences in TSPO occupancy and Ki values of ONO-2952 across the brain, as demonstrated by the curve fit analysis of pharmacokinetic-TSPO occupancy in each ROI. Distribution of TSPO occupancy within regions involved in stress signaling, including the amygdala, hippocampus, and medial frontal cortex support our hypothesis that ONO-2952 attenuates the stress response in humans by acting on central TSPO. These findings indicate that ONO-2952 is a promising candidate for the treatment of stress-related disorders.

Disclosures: **G.W. Frankle:** 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.; ONO Pharmaceutical Co., Ltd. **R. Narendran:** 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.; ONO Pharmaceutical Co., Ltd. **S.N. Mason:** 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.; ONO Pharmaceutical Co., Ltd. **A.T. Wood:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd. **F. Suto:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd. **K. Mitsui:** A. Employment/Salary (full or part-time):; ONO Pharmmaceutical Co., Ltd. **M. Kobayashi:** A. Employment/Salary (full or part-time):; ONO Pharmmaceutical Co., Ltd. **T. Ohno:** A. Employment/Salary (full or part-time):; ONO Pharmmaceutical Co., Ltd. **A. Yamauchi:** A. Employment/Salary (full or part-time):; ONO Pharmmaceutical Co., Ltd. **M. Komeno:** A. Employment/Salary (full or part-time):; ONO Pharmmaceutical Co., Ltd. **S. Katsumata:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical Co., Ltd..

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.05/BB22

Topic: C.19. Drug Discovery and Development

Support: CIC, UMSNH 160

Title: Isobolographic Analyses of Memantine-Metamizol and Memantine-Ketoprophen antinociceptive oral combinations in rat formalin test

Authors: A. ZUÑIGA-ROMERO[1], M. Y. GAUTHEREAU TORRES[2], *L. F. ORTEGA-VARELA[3];
[1]Facultad de Químico Farmacobiología, [2]2Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Univ. Michoacana de San Nicolás de Hidalgo, Morelia, Mexico; [3]UMSNH, Lic. En Salud Publica, Morelia, Mexico

Abstract: This study was achieved to assess possible synergistic interaction between Memantine-Metamizol and Memantine-Ketoprophen orally administered in the rat model of formalin test. Female Wistar rats (200-350 g) were injected into the dorsal surface of the right hind paw with 50 μg of 1% formalin. This substance induced a flinching pain-related behavior, the reduction of such behavior is considered as antinociception. The percent of antinociceptive effect was determined by the oral administration of Memantine (10-80 mg/kg), Metamizol (30-600 mg/kg), Ketoprophen (30-600 mg/kg) and the combinations (Memantine-Metamizol and Memantine-Ketoprophen). To analyze the nature of the interaction, isobolographic analysis was used in a fixed-dose radio (0.5:0.5), on the basis of their ED30 values: Memantine (78.55 ± 3.5 mg/kg), Metamizol (139.49 ± 6.2 mg/kg) and Ketoprophen (92.16 ± 8.9 mg/kg). The combination of Memantine-Metamizol and Memantine-Ketoprophen significantly reduced the number of flinches in the second phase of the formalin test. For the isobolographic analyses, the theoretical effective dose 30 for the Memantine-Metamizol combination (ED30 T) was 109.02 ± 4.8 mg/kg. Experimentally, the effective dose 30 (ED30 E) was significantly lower (90.95 ± 3.30 mg/kg). In the same sense, the theoretical effective dose 30 for the Memantine-Ketoprophen combination (ED30 T) was 85.36 ± 5.8 mg/kg. Experimentally, the effective dose 30 (ED30 E) was significantly lower (52.82 ± 6.8 mg/kg), indicating the presence of synergism for both combinations. Results indicate that oral administration of Memantine-Metamizol and Memantine-Ketoprophen combinations can interact synergistically to reduce inflammatory pain in the formalin test.

Disclosures: A. Zuñiga-Romero: None. **L.F. Ortega-Varela:** None. **M.Y. Gauthereau Torres:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.06/BB23

Topic: C.19. Drug Discovery and Development

Title: The inhibition of N-acylethanolamine acid amidase activity produces antinociceptive effect

Authors: *O. SASSO, A. REGGIANI, D. PIOMELLI;
Fondazione Inst. Italiano Di Tecnologia, Genoa, Italy

Abstract: The amides of fatty acids with ethanolamine (fatty acid ethanolamides [FAEs]) are a family of lipid-derived messengers that participate in the control of multiple physiological functions, including pain and inflammation. Saturated or monounsaturated members of this family, such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), are produced in innate immune and neural cells by the action of a selective phospholipase, N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD), and exert antinociceptive and anti-inflammatory effects in experimental animals and humans. Such effects are primarily, albeit not exclusively and, due to the ability of PEA and OEA to engage the ligand-activated transcription factor, peroxisome proliferator-activated receptor-α (PPAR-α) and, to which they bind with high affinity. The actions of these lipid messengers are terminated by enzyme-mediated hydrolysis, which is catalyzed by 2 intracellular lipid amidases: N-acylethanolamine acid amidase (NAAA) and fatty acid amide hydrolase (FAAH) . Although NAAA and FAAH share the ability to cleave lipid amide bonds, they differ markedly in primary structure, substrate selectivity, and cellular localization. Proinflammatory stimuli suppress FAE production and causing a reduction in the cellular levels of these anti-inflammatory lipid mediators. The latter response is reversed by pharmacological blockade of NAAA-mediated FAE hydrolysis, suggesting that inhibition of intracellular NAAA activity might represent a novel mechanistic approach to control inflammation. Although there is a growing appreciation for the role of NAAA in the control of inflammation, it is still unknown whether the ability of this enzyme to terminate FAE signaling might also contribute to pain regulation. To address this question, in the present study we used the compound ARN077 (5-phenylpentyl N-[(2S,3R)-2-methyl-4-oxo-oxetan-3-yl] carbamate), which we recently identified as a highly potent and selective inhibitor of human NAAA (IC50 approximately 7 nM). Our results show that local administration of ARN077 normalizes FAE levels in inflamed mouse skin and in ligated nerve and blunts the hyperalgesia and allodynia evoked in mice by carrageenan and sciatic nerve injury and in rats by ultraviolet B (UVB) radiation.

Disclosures: O. Sasso: None. **A. Reggiani:** None. **D. Piomelli:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.07/BB24

Topic: C.19. Drug Discovery and Development

Title: Neurochemical and behavioural assessment of tapentadol alone and in combination with donepezil for the memory impairment in rats

Authors: *V. BENADE, S. DARIPELLI, G. BHYRAPUNENI, R. PONNAMANENI, A. MANOHARAN, V. GOURA, R. GADI, R. NIROGI;
Suven Life Sci. Ltd, Hyderabad, India

Abstract: Tapentadol is a norepinephrine reuptake inhibitor (NRI) and μ-opioid receptor (MOR) agonist. NRIs are reported to have pro-cognitive property by increasing norepinephrine and acetylcholine (ACh) levels. However, phase 2/3 clinical reports indicate memory impairment in patients taking tapentadol. Tapentadol is indicated for the chronic pain and there is a possibility of co-prescription of cholinergic compounds to manage the symptoms of memory impairment. Based on this, the objective of the present investigation was to evaluate the neurochemical involvement in the memory impairment observed with tapentadol. To study the effects of combination of donepezil with tapentadol, microdialysis and memory assessment was carried out. Brain microdialysis was carried out to evaluate the effect of tapentadol on ACh levels in prefrontal cortex (PFC) and contextual fear conditioning (CFC) model to evaluate the effect on memory. In microdialysis, tapentadol produced 2-3 fold increase in cortical ACh levels and the increase was attenuated by SCH-23390, a D1 antagonist indicating the involvement of NRI property in ACh modulation. In combination with donepezil, tapentadol produced significant increase in ACh levels compared to either treatment alone. In CFC model, tapentadol alone produced memory impairment which was blocked by MOR antagonist, naltrexone. However, combination of donepezil had no effect on memory impairment observed with tapentadol. Results from the present investigation indicate that the memory impairment with tapentadol might be mediated through the MOR and no beneficial effects were observed in combination with donepezil in preclinical model of memory assessment.

Disclosures: **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **S. Daripelli:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **R. Ponnamaneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **A. Manoharan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **V. Goura:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **R. Gadi:** A.

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Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

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Program#/Poster#: 714.08/BB25

Topic: C.19. Drug Discovery and Development

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UVM Neuroscience Cobre molecular biology and imaging facilities

Totman Medical Research Trust Fund

Peter Martin Aneurysm endowment

Title: Differential mechanisms of vasodilation of PACAP and CGRP in rat middle meningeal artery: Potential role in migraine headache

Authors: A. SYED[1], V. MAY[2], *G. C. WELLMAN[3];
[2]Neurolog. Sci., [3]Dept. of Pharmacol., [1]Univ. of Vermont, Burlington, VT

Abstract: Migraine is the most common incapacitating neurological disorder, characterized by an intense pulsating headache. The cellular mechanisms contributing to migraine headache are poorly understood, but a leading hypothesis is that prolonged dilation of cranial arteries, specifically the middle meningeal artery (MMA), is involved in the sensation of headache pain. The neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and calcitonin gene-related peptide (CGRP) have been shown to potently dilate the MMA and induce migraine-like headaches. The mechanisms by which these peptides exert their effect on the MMA remain unclear. The goal of this study is to decipher the mechanisms of PACAP and CGRP-induced dilations using freshly isolated pressurized rat MMAs. At an intravascular pressure of 40 mmHg, MMAs developed myogenic tone (i.e. constricted) representing ~ 40 % decrease in diameter. Treatment of these arteries with PACAP (3 nM) or CGRP (1 nM) caused significant vasodilation (59 ± 5.8 % of maximum and 77 ± 4.01 % of maximum, respectively). PACAP-induced dilation was abolished in the presence of glibenclamide, an ATP-sensitive K^+ (K_{ATP}) channel blocker.

However, CGRP-induced dilation remained unaffected by treatment with glibenclamide, alone. Paxilline, a blocker of large-conductance Ca^{2+}-activated K^+ (BK) channels, or 4-aminopyridine, a blocker of voltage-gated K^+ (K_V) channels also did not affect CGRP-induced MMA dilation. Further, CGRP-induced dilations were not altered by a combination of L-nitroarginine (L-NNA) to inhibit nitric oxide synthesis, indomethacin to inhibit prostacyclin synthesis, apamin to block endothelial small-conductance Ca^{2+}-activated K^+ channels and TRAM-34 to block endothelial intermediate-conductance Ca^{2+}-activated K^+ channels. Interestingly, CGRP-induced dilations were blocked by raising extracellular K^+ to 30 mM, implicating involvement of K^+ channel activation in the dilatory response of this peptide. Further, CGRP-induced dilations were abolished by a combination of compounds that included glibenclamide, paxilline, L-NNA, indomethacin, apamin, TRAM-34 and thapsigargin, an inhibitor of the sarco-endoplasmic reticulum Ca^{2+}-ATPase. In summary, although PACAP and CGRP have been reported to increase adenylyl cyclase activity, they act via distinct vasodilatory mechanisms in the MMA. PACAP induces vasodilation through K_{ATP} channel activation, while CGRP appears to utilize multiple cell signaling pathways. By understanding the distinct mechanisms involved in MMA dilation caused by PACAP and CGRP it may be possible to develop new combination therapies for migraine headache.

Disclosures: A. Syed: None. **V. May:** None. **G.C. Wellman:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.09/BB26

Topic: C.19. Drug Discovery and Development

Support: NSC 102-2311-B-002-034-MY3

NHRI-EX103-10104NI

Title: Gabapentin reverses central hyperactivities and connectivities, and suppresses medial prefrontal cortical glucose metabolism in rats with neuropathic pain

Authors: H.-C. LIN[1], Y.-H. HUANG[2], T.-H. CHAO[1], W.-Y. LIN[2], W.-Z. SUN[2], *C.-T. YEN[1]; [1]Life Sci., Natl. Taiwan Univ., Taipei, Taiwan; [2]Anesthesiol., Natl. Taiwan Univ. Hosp, Taipei, Taiwan

Abstract: Gabapentin (GBP) is known to suppress neuropathic hypersensitivity of primary afferents and the spinal cord dorsal horn neurons. However, it is unclear how GBP affects neuronal activities in the brain. In this study we identify the brain regions where GBP changes the brain glucose utilization at the effective dose that alleviates mechanical allodynia. We used the spared nerve injury (SNI) of the sciatic nerve as the neuropathic pain model. Mechanical allodynia was verified using the von Frey filaments test. 18F-fluorodeoxyglucose-positron emission tomography (FDG-PET) scanning was performed 3 times in each rat to measure the change of glucose metabolism in the brain before SNI, after SNI, and after GBP (100 mg/kg, i.p.) treatment. For each PET scanning, dynamic mechanical allodynic stimuli were administered during the FDG uptake period, and the efficacy of GBP treatment was confirmed. Comparing the PET imaging data before and after the GBP treatment, the SNI-induced increases of glucose metabolism in the thalamus and medial cerebellum were reversed, and a significant decrease occurred in glucose metabolism in the medial prefrontal cortex (mPFC), including the anterior cingulate cortex. GBP treatment also reversed post-SNI connectivity increases among limbic cortices, basal ganglia, and thalamus. Immunohistochemical analysis of c-Fos expression showed that SNI and GBP treatment induced changes in neuronal activities, not glial cells. Our results indicate that GBP analgesic effect may be mediated by reversing central hyperactivity and connectivity, and suppressing mPFC activity in the brain.

Disclosures: H. Lin: None. **C. Yen:** None. **T. Chao:** None. **Y. Huang:** None. **W. Lin:** None. **W. Sun:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.10/BB27

Topic: C.19. Drug Discovery and Development

Title: Development of a cathepsin S inhibitor for neuropathic pain: Efficacy in a preclinical model of taxol-induced neuropathy and phase 1 clinical profile

Authors: *L. J. HOLSINGER[1], A. MARQUEZ[2], T. MIXCOATL-ZECUATL[2], D. B. KARPF[1], A. SUDHAKAR[1], J. M. DENER[1], K. ELROD[1], R. F. G. BOOTH[1], N. A. CALCUTT[2]; [1]Virobay Inc., Menlo Park, CA; [2]Dept. of Pathology, Univ. of California San Diego, La Jolla, CA

Abstract: Cathepsin S is a cysteine protease known to be critical in the development of neuropathic pain. Following neuronal injury, membrane bound fractalkine (FKN) is expressed at the surface of injured neurons. Also following injury cathepsin S is released from resident immune cells, including microglia and macrophages, where it cleaves membrane bound FKN allowing the release of the pro-nociceptive chemokine soluble FKN. FKN-mediated recruitment and activation of microglia and other cells involved in immune defense triggers release of pro-inflammatory cytokines and other inflammatory mediators, which then propagate an exaggerated pain response. Cathepsin S lies at the intersection of neuronal-immune communication now recognized as critical in the development of chronic pain. We have demonstrated efficacy of two selective cathepsin S inhibitors, VBY-036 and VBY-285, in rodent models of taxol-induced neuropathic pain. VBY-036 and VBY-285 are potent inhibitors with high selectivity for cathepsin S. Both compounds are efficacious at reversing established tactile allodynia which had been induced by repeated taxol administration. VBY-285 was efficacious in reversing established tactile allodynia in a rat model after a single dose. Maximal efficacy, equal to that observed after gabapentin administration, was achieved with repeated once-a-day dosing. No tolerance to repeated dosing was observed and, unlike gabapentin, cathepsin S inhibition produces no sedative effects. Cessation of VBY-285 dosing resulted in a slow return of allodynia, with analgesic efficacy re-established when daily dosing was resumed. VBY-036 was also efficacious in reversing established tactile allodynia in a mouse model after taxol dosing. VBY-036 administration started prior to taxol administration resulted in a greater level of analgesic efficacy in a shorter time frame than administration following the establishment of allodynia. Virobay is progressing VBY-036 in human clinical studies in neuropathic pain. Results of these studies and a VBY-036 Phase 1 single-ascending dose and multiple-ascending dose studies in healthy volunteers will be presented where the safety and pharmacokinetics of VBY-036 oral administration were evaluated. VBY-036 was well tolerated with plasma pharmacokinetics exhibiting roughly dose-proportional exposures. Target engagement on cathepsin S was detected in PBMCs using a pharmacodynamic biomarker, identifying plasma exposures required for maximal cathepsin S inhibition. VBY-036 is in development as a treatment for neuropathic pain, with a planned proof-of-concept Phase 2a study in chronic chemotherapy-induced peripheral neuropathy.

Disclosures: **L.J. Holsinger:** A. Employment/Salary (full or part-time):; Virobay Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Virobay Inc.. **A. Marquez:** None. **T. Mixcoatl-Zecuatl:** None. **D.B. Karpf:** None. **A. Sudhakar:** None. **J.M. Dener:** None. **K. Elrod:** None. **R.F.G. Booth:** None. **N.A. Calcutt:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.11/BB28

Topic: C.19. Drug Discovery and Development

Support: PSVT-3, MDEIE, Quebec, Canada

Title: Selective Melatonin MT2 receptor ligands decrease neuropathic pain through modulation of brainstem descending antinociceptive pathways

Authors: *G. GOBBI[1], M. LOPEZ-CANUL[1,2], E. PALAZZO[3], L. LUONGO[3], S. DOMINGUEZ-LOPEZ[1], B. LACOSTE[1], V. GRANADOS-SOTOS[4], S. MAIONE[3]; [1]McGill Univ., Montreal, QC, Canada; [2]Univ. Veracruzana, Xalapa, Mexico; [3]Second Univ. of Naples, Naples, Italy; [4]Cinvestav Sede Sur, México D.F., Mexico

Abstract: Background: Neuropathic pain is a major public health problem, which results in personal suffering, reduced productivity and substantial costs for health system. Only a few treatments are available for this condition causing several side effects. Double-blind clinical studies have reported that melatonin, a neurohormone acting on the two G-protein receptor MT1 and MT2, can be an effective treatment for pain. Here we have tested the antinociceptive effects melatonin and two selective MT2 receptor partial agonists N-{2-[(3-methoxyphenyl)phenylamino]ethyl}acetamide (UCM765) and N-{2-[(3-bromophenyl)-4-fluorophenylamino]ethyl}acetamide (UCM924) in two animal models of neuropathic pain. Methods: Rat spinal L5-L6 nerve ligation and spared nerve injury models were used to test antiallodynic effects after subcutaneous injection of increasing doses of UCM765 and UCM924. The effects were compared to melatonin (150 mg/kg) and gabapentine (100 mg/kg). *In vivo* electrophysiology combined with tail flick test was used to record electrical activity of ON and OFF neurons in the rostral ventromedial medulla (RVM) following microinjection of UCM765 and UCM924 in the rostral ventrolateral periaqueductal gray (vlPAG). Motor impairments effects were assessed using the Rotarod test. Results and discussion: UCM765 and UCM924 decrease allodynia in both rat spinal L5-L6 nerve ligation and spared nerve injury models. These effects are 1) dose-dependent and blocked by the selective MT2 receptor antagonist 4P-PDOT, 2) superior to high doses of melatonin and comparable to gabapentin, but 3) without noticeable motor-sedative effects in the rotarod test. *In vivo* electrophysiology combined to tail flick test indicate that microinjection of UCM765 and UCM924 in the vlPAG decreases tail flick responses, depressing the firing activity of ON cells and activating the firing of OFF cells, with a MT2 receptor-dependent effect. Altogether, these data demonstrate that MT2 receptor ligands have analgesic properties through modulation of brainstem descending antinociceptive pathways.

Disclosures: **G. Gobbi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents on novel melatonin selective ligands and their use on pain. **M. Lopez-Canul:** None. **E. Palazzo:** None. **L. Luongo:** None. **S. Dominguez-Lopez:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent on melatonin ligands and pain. **B. Lacoste:** None. **V. Granados-Sotos:** None. **S. Maione:** None.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

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Program#/Poster#: 714.12/BB29

Topic: C.19. Drug Discovery and Development

Support: National Science Council of Taiwan (NSC102-2325-B-110-002)

Kaohsiung Armed Forces General Hospital, Taiwan (102-08; 103-08)

Title: Involvement of spinal transforming growth factor-beta 1 in the analgesic effects of a coral-derived compound flexibilide in neuropathic rats

Authors: *S.-Y. HUANG[1], C.-H. CHEN[2], N.-F. CHEN[3], C.-W. FENG[2], H.-C. HUNG[2], Y.-Y. LIN[1], P.-J. SUNG[4], C.-S. SUNG[5], S.-N. YANG[6], H.-M. WANG[7], J.-H. SHEU[1], W.-F. CHEN[8], Z.-H. WEN[1];
[1]Dept. of Marine Biotech. and Resources, Asia-Pacific Ocean Res. Ce, Natl. Sun Yat-Sen Univ., Kaohsiung, Taiwan; [2]Doctoral Degree Program in Marine Biotech., Natl. Sun Yat-sen Univ. and Academia Sinica, Kaohsiung, Taiwan; [3]Div. of Neurosurgery, Dept. of Surgery, Kaohsiung Armed Forces Gen. Hosp., Kaohsiung, Taiwan; [4]Taiwan Coral Res. Ctr., Natl. Museum of Marine Biol. and Aquarium, Pingtung, Taiwan; [5]Dept. of Anesthesiol., Taipei Veterans Gen. Hosp., Taipei, Taiwan; [6]Dept. of Pediatrics, E-DA Hosp., I-Shou Univ., Kaohsiung, Taiwan; [7]Dept. of Fragrance and Cosmetic Science, Ctr. of Excellence for Envrn. Med., Kaohsiung Med. Univ., Kaohsiung, Taiwan; [8]Dept. of Neurosurg., Kaohsiung Chang Gung Mem. Hosp. and Chang Gung Univ. Col. of Med., Kaohsiung, Taiwan

Abstract: Chronic neuroinflammation has a key role in the development and maintenance of neuropathic pain. Flexibilide, which can be obtained from cultured soft coral, has peripheral anti-inflammatory and analgesic effects in carrageenan-injected rats. The antinociceptive effects of flexibilide in neuropathic pain have not been previously reported. In this study, we investigated

and further characterized the antinociceptive properties of flexibilide in the chronic constriction injury (CCI) model of neuropathic rats. At 14 days after surgery, we found that a single intrathecal (i.t.) injection of flexibilide significantly attenuated CCI-induced thermal hyperalgesia. I.t. 10-micro g flexibilide twice daily prevented both the development of thermal hyperalgesia and weight-bearing deficits in CCI rats. On the ipsilateral dorsal horn of the lumbar spinal cord, i.t. flexibilide significantly inhibited CCI-induced glial cell activation (activation of microglia and astrocytes) and the upregulated pro-inflammatory protein inducible nitric oxide synthase. Furthermore, at 14 days after surgery, i.t. flexibilide attenuated downregulation of spinal transforming growth factor-beta 1 (TGF-beta 1) in CCI rats. Finally, i.t. SB431542 (a selective inhibitor of TGF-beta type I receptor) blocked the analgesic effects (anti-thermal hyperalgesia and anti-weight-bearing deficits) of flexibilide in CCI rats. Our results indicate that flexibilide may be a therapeutic agent for neuropathic pain, and spinal TGF-beta 1 may participate in the analgesic effects of flexibilide.

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Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.13/BB30

Topic: C.19. Drug Discovery and Development

Title: Addition of a single methyl group converts a small molecule Nav channel inhibitor targeting the voltage sensor into a Nav channel “activator”

Authors: L. WANG, S. ZELLMER, D. PRINTZENHOFF, M. CHAPMAN, *N. CASTLE; Neusentis (Pfizer), Durham, NC

Abstract: A structural class of aryl sulfonamide human Nav1.3 or Nav1.7 voltage gated sodium channel inhibitors that interact with the domain 4 voltage sensor region was recently described (McCormack et al, PNAS 2013 110:E2724-32). Included in this class is PF-05661014, which selectively inhibits hNav1.3 (IC_{50} 1.6 μM, compared to IC_{50} of >30 μM for hNav1.7). As with other members of this inhibitor class, PF-05661014 interacts preferentially with the inactivated state of the channel. However, when a single methyl group was added to the urea linker of this compound to form PF-06526290, it was found to greatly slow the rate of Nav1.3 inactivation

(tau_{inact} = 1 ms, control, tau_{inact} = 100 ms PF-06526290, EC_{50} 2 μM). However, maintaining depolarization to promote inactivation results in subtype selective channel inhibition of Nav1.3 similar to that seen with the desmethyl compound PF-05661014. Interestingly, the ability of PF-06526290 to slow inactivation was observed for all Nav channel subtypes tested (i.e. Nav1.2, Nav1.5, Nav1.7, and Nav1.8). By examining Nav1.7, which exhibited no sensitivity to inhibition by PF-06526290, we observed that while inactivation is slowed in the presence of the compound, as inactivation progresses, the compound appears to be functionally displaced. Nav1.7 currents elicited following a short hyperpolarizing step to allow recovery from inactivation exhibit normal fast inactivation. However, if the recovery period of the hyperpolarizing step is increased, the slowing of inactivation redevelops with a rate that accelerates with increasing compound concentration. The PF-06526290 induced slowing of inactivation seems to be mechanistically distinct from the subtype selective inhibition seen with this agent and it desmethyl analog. A previously characterized mutation of the domain 4 voltage sensor of either Nav1.3 or Nav1.7 (called M123) was found to modulate the inhibitory effects of PF-06526290, but had no obvious effect on compound induced slowing of inactivation of either channel subtype. The ability of PF-06526290 to delay Nav channel inactivation is similar to that seen with alpha scorpion toxins. When PF-06526290 and the alpha toxin, Lqh3 were applied together, slowing of inactivation appeared to be synergistic, suggesting separate interaction sites on the channel. In conclusion, the current study has shown that a single methyl substitution on a molecule can introduce additional modalities of interaction and modulation of Nav channel function.

Disclosures: **L. Wang:** A. Employment/Salary (full or part-time):; Pfizer Inc. **S. Zellmer:** A. Employment/Salary (full or part-time):; Pfizer Inc. **D. Printzenhoff:** A. Employment/Salary (full or part-time):; Pfizer. **M. Chapman:** A. Employment/Salary (full or part-time):; Pfizer Inc. **N. Castle:** A. Employment/Salary (full or part-time):; Pfizer Inc.

Poster

714. New Drugs for Pain, Headache, and Migraine

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Topic: C.19. Drug Discovery and Development

Support: NIEHS grant R01 ES002710

NIH CounterAct

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Louisiana Governors' Biotechnology Initiative

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Title: Newly designed potent small molecule inhibitors of soluble epoxide hydrolase (sEH) target chronic inflammatory & neuropathic pain

Authors: *W. K. SCHMIDT[1], K. S. S. LEE[2], K. M. WAGNER[2], B. INCEOGLU[2], B. D. HAMMOCK[2];
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Abstract: Arachidonic acid is a substrate for three major enzymatic routes of metabolism by cyclooxygenase, lipoxygenase and cytochrome P450 enzymes that convert polyunsaturated fatty acids to potent pharmacologically active lipids including prostanoids, leukotrienes and epoxyeicosatrienoic acids (EETs). While the first two groups of lipids are largely pro-inflammatory molecules that sensitize or activate neurons to painful stimuli, the EETs reduce the excitability of neurons and produce potent analgesic and anti-inflammatory activity *in vivo*. The soluble epoxide hydrolase (sEH, EC 3.3.2.10) metabolizes endogenously derived EETs to their corresponding diols, the dihydroxyepoxyeicosatrienoic acids (DHETs), which in turn have substantially less biological activity. Inhibition of sEH results in increases endogenous levels of EETs which produce significant beneficial effects in multiple pathobiologies involving pain, inflammation, and cardiovascular and respiratory function. sEH has emerged as a pharmaceutical target for hypertension, inflammation, organ-protection and, more recently, chronic pain including neuropathic pain. It is estimated that half of diabetic patients will suffer from painful diabetic neuropathy (PDN); current treatments for PDN have limited efficacy and often produce treatment-limiting side effects. Thus, alternate therapeutic strategies are needed. To better study the effects of epoxy-fatty acids and investigate the anti-nociception *in vivo*, a new series of N,N'-disubstituted urea-based sEH inhibitors has been synthesized that have dramatically increased potency, high target occupancy, better water solubility, and improved pharmacokinetic profiles. The PK/ADME profiles of these compounds and their efficacy in animal models of diabetic neuropathic pain is described in this report. The new inhibitors display improved efficacy in relieving pain-related behaviors in mouse and rat diabetic neuropathic pain models. sEH inhibitors have also demonstrated dramatic analgesic effects vs. pathological inflammatory and neuropathic pain in cats and dogs (osteoarthritis) and in equine laminitis after all conventional treatments had failed, demonstrating that these effects extend to multiple species. In summary, the results suggest that sEH inhibitors could be attractive additions to pharmacological treatment of chronic painful conditions in humans.

Disclosures: **W.K. Schmidt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EicOsis, LLC. **K.S.S. Lee:** None. **K.M. Wagner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EicOsis, LLC. **B. Inceoglu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EicOsis, LLC. **B.D. Hammock:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EicOsis, LLC.

Poster

714. New Drugs for Pain, Headache, and Migraine

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 714.15/BB32

Topic: C.19. Drug Discovery and Development

Title: Evaluation in the modulation of brain and spinal cord monoamine levels on the anti-nociceptive activity of tapentadol

Authors: S. DARIPELLI, *S. M. IRAPPANAVAR, G. BHYRAPUNENI, V. BENADE, G. AYYANKI, R. PONNAMANENI, A. MANOHARAN, R. NIROGI;
DISCOVERY RESEARCH, DMPK, SUVEN LIFE SCIENCES LTD, HYDERABAD, India

Abstract: Role of cortical dopamine (DA) and norepinephrine (NE) modulation in emotional and pain processing is well known. In addition, these monoamines are involved in the modulation of pain perception in spinal cord. Tapentadol, a drug with dual action has norepinephrine (NE) reuptake blocking and μ-opioid agonistic activity and is recently been introduced for the treatment of moderate to severe pain. Objective of the present study was to examine the effects of tapentadol on modulation of monoamines in prefrontal cortex (PFC) and dorsal horn. For brain microdialysis, tapentadol was administered intraperitoneally at 4.64, 10 or 21.5 mg/kg to male Wistar rats. Based on the results from brain microdialysis, 10 mg/kg i.p. was chosen as the dose for spinal microdialysis in freely moving rats. Tapentadol produced significant and dose dependent increase in DA and NE levels of PFC with no effect on cortical serotonin (5-HT) levels. However in dorsal horn, 5-HT levels were significantly increased in addition to DA and NE after administration at 10 mg/kg, i.p. Although the density of DAT is low in PFC, the increase of DA levels in PFC could be mediated through the inhibition of NE transporter. Increase in 5-HT levels of dorsal horn could be mediated through the activation of μ-

opioid receptor. The results from the present investigation suggest that clinical efficacy of tapentadol in neuropathic pain state is mediated through the enhanced 5-HT levels in dorsal horn and dopamine levels in prefrontal cortex in addition to the increase in norepinephrine levels.

Disclosures: **S. Daripelli:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **S.M. Irappanavar:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **G. Ayyanki:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **R. Ponnamaneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **A. Manoharan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India.

Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 715.01/CC1

Topic: C.21.Stroke Recovery

Support: Loyola Neuroscience Research Institute

Department of Veterans Affairs

Title: Aged rat sex differences in learning sensorimotor tasks and post-stroke recovery

Authors: V. J. BORKOWSKI[1], *S.-Y. TSAI[2], K. S. HSU[2], A. E. MARINOPOULOS[2], V. A. HUSAK[2], C. M. PAPADAPOULOS[2], G. L. KARTJE[2];
[1]Neurosci. Institute, Loyola Univ. Chicago Hlth. Sci. Div., Maywood, IL; [2]Edward Hines Jr VA Hosp., Hines, IL

Abstract: Sex-based differences in learning and recovery from stroke are an area of research not yet fully explored, especially with regard to aged rats performing sensorimotor tasks. In the human population, post-menopausal females have been shown to recover from stroke less successfully than males, and therefore studying sex differences in rat models of stroke is

clinically relevant. 18 month-old Fischer 344 male and female aged rats were used for these studies. Rats were divided into the following groups: ovariectomized (OVX) females, intact females, sham OVX males, and intact males. Pre-stroke, rats were trained in the skilled forelimb reaching task and skilled ladder walk test. The rats then underwent focal ischemic stroke via middle cerebral artery occlusion to affect the sensorimotor cortex associated with the preferred forelimb. Rats were then tested on the behavioral tests for eight weeks to assess post-stroke recovery. At the end of the eight weeks, rats were sacrificed for Golgi-Cox staining and dendritic characterization. Our results show that pre-stroke intact females began reaching earlier ($\mu = 2 \pm 1$ days vs. male $\mu = 7 \pm 1$ days, $p < 0.05$), had their first successful reach earlier ($\mu = 3 \pm 1$ days vs. male $\mu = 8 \pm 1$ days, $p < 0.05$), and reached baseline success scores earlier than their aged male counterparts ($\mu = 14 \pm 2$ days vs. male $\mu = 18 \pm 1$ days, $p < 0.05$). OVX females had the slowest times in all categories. In learning the skilled ladder walk task pre-stroke, intact females reached baseline earlier than males ($\mu = 4 \pm 1$ days vs. male $\mu = 8 \pm 2$ days, $p < 0.05$) and earlier than OVX females. Following stroke, a sex difference was seen in the skilled forelimb reaching task with all male groups recovering faster than all female groups with the OVX females recovering worse. No difference was seen between post-stroke OVX and intact females in the skilled ladder rung walking task, but males improved better than females. Lesion analysis revealed no significant difference in stroke lesion size, thus indicating that lesion size did not account for the differences in post-stroke recovery. In conclusion, our results show that aged intact female rats learn sensorimotor tasks faster than age-matched males and age-matched OVX females pre-stroke. Females had worse post-stroke recovery than males, with OVX females performing worse than all other groups in the skilled reaching task. This suggests an estrogen effect on post-stroke long-term sensorimotor recovery. Neuronal dendritic analysis of pertinent brain areas involved in sensorimotor recovery is currently underway. This study was funded by the Loyola Neuroscience Research Institute and the Department of Veterans Affairs.

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Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 715.02/CC2

Topic: C.21.Stroke Recovery

Title: Influence of sildenafil citrate on development of local cerebral infarction

Authors: *M. NEBIERIDZE, I. ERKOMAISHVILI, G. AZIKURI, M. DEVDARIANI, N. SIKHARULIDZE, N. MITAGVARIA;
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Abstract: The goal of this study was investigation of development of the local infarction in the brain cortex elicited by the photochemically induced arterial thrombosis, on the background of Sildenafil Citrate administration. Experiments were carried out in the adult male albino rats, weighing 220 - 250 grams. The animals were divided into experimental and control groups. Under the light chloral hydrate anesthesia (0,4 g/kg, i.p.) local vascular thrombosis was induced in the left frontal lobe of the rat brain (Control group). With this purpose animal was intravenously injected with the photosensitive biological stain Rose Bengal, and than the left frontal lobe region was illuminated, for one hour, with the halogen light beam (via the glass-fiber transducer). Following this procedure the light source was transferred onto the right hemisphere and symmetrical part of the brain was illuminated. In other words, the frontal lobe infarction was produced bilaterally, with one-hour delay, in left and right hemispheres of the brain. The above procedure was repeated in the experimental group of the animals with the following modification - 15 minutes after beginning of the right hemisphere illumination the animal was injected with Sildenafil Citrate (0.01 g/kg, i.p.), the action maximum of which was attained at the end of the right hemisphere illumination. Four hours following the illumination cessation the animals were perfused transcardially with 10% formalin solution, and 100 μm-thin frontal slices of the brain were prepared. By means of light microscopy the geometric indices of the lesioned region (volume of infarction – V; area measured in the lesion focus observed on the frontal section – S) were evaluated. The values of the above indices, obtained in the control and experimental animals, are presented in the Table below.

	Left hemisphere		Right hemisphere	
Animal group				
	V (mm^3)	S (mm^2)	V (mm^3)	S (mm^2)
Control	30.25±2.9	10.22±1.9	27.8±3.1	9.25±1.1
Experimental	24.9±3.2	8.25±1.2	37.45±3.5	11.85±1.7

The data indicate that, on the background of Sildenafil Citrate, geometrical parameters of the developed infarction statistically significantly exceed those of infarction without the preparation impact. Probable mechanisms of this difference are discussed in the presentation.

Disclosures: M. Nebieridze: None. **I. Erkomaishvili:** None. **G. Azikuri:** None. **M. Devdariani:** None. **N. Sikharulidze:** None. **N. Mitagvaria:** None.

Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 715.03/CC3

Topic: C.21.Stroke Recovery

Title: Hormetic effect and its distribution at hydrogen peroxide-induced oxidative stress

Authors: *M. DEVDARIANI, L. DAVLIANIDZE, M. NEBIERIDZE, L. GUMBERIDZE, I. KVACHAKIDZE, N. MITAGVARIA;
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Abstract: The increase in resistance to oxidative stress is associated with extension of the vitality. In particular, low doses of oxidative stress can slow the aging process. Here we have a deal with a phenomenon which is known as "Hormesis". This term describes phenomenon, when in response to low doses of toxins or any other stressors, the body develops positive reaction - an adaptive stress response, which provides stability of cells to higher doses of stressogenic factors. The main purpose of this study was to reveal a behavioral manifestation of hormetic effect in white rats caused by Hydrogen Peroxide (H2O2)-induced oxidative stress. The group of rats instead of regular drinking water for four weeks received 0.1% (first group) or 0.2% (second group) solution of H2O2; since the beginning of fifth week the animals began training in the multiway maze to learn optimal trajectory for getting into the nest-box. Prior to the completion of testing (7-10 days), rats, instead of drinking water continued to take a solution of H2O2. It was found that animals exposed to 0.1% H2O2 significantly increased their behavioral activity (in comparison with the control ones) and at the end of the seventh day they unmistakably passed the maze almost twice as fast as the control animals (well pronounced hormetic effect), but in case of 0.2% - we observed sharp disruption of this effect on the 7-8 days of training. Possible mechanisms causing this kind of behavior are discussed.

Disclosures: M. Devdariani: None. **L. Davlianidze:** None. **M. Nebieridze:** None. **L. Gumberidze:** None. **I. Kvachakidze:** None. **N. Mitagvaria:** None.

Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

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Program#/Poster#: 715.04/CC4

Topic: C.21.Stroke Recovery

Support: NIH Grant F32NS078933 (SM)

NIH Grant R01NS046400 (SD)

Title: PGF2a-FP receptor Role in a mouse model of intracerebral hemorrhage

Authors: *S. MOHAN[1], E. KOLLER[1], S. NARUMIYA[2], S. DORE[1];
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Abstract: Prostaglandin F2a (PGF2a) has been described to exert beneficial and detrimental effects in various neurological disorders. Brain damage following an intracerebral hemorrhagic (ICH) stroke is associated with the release of inflammatory molecules such as PGF2a; however, the role of PGF2a and its cognate FP receptor in ICH remains unclear. Using age (2.5-3.5 months) and weight-matched (20-30g) adult male WT and FP-/- C57BL/6 single unilateral intrastriatal injection of collagenase VII-S [0.03 Units in 0.2µL saline] was given using specific stereotaxic coordinates. At 24, 48 and 72h after collagenase injection, neurological and functional outcomes were scored, and brains were harvested for hemorrhage injury analysis at 72h. The following neurobehavioral deficit assays were performed: (a) neurological deficit on a 24 point scale, (b) rotarod performance and (c) forepaw grip strength test. The following histopathological analysis was performed: (a) Cresyl violet, (b) GFAP and Iba1 immunoreactivity and (c) Perls' (ferric Fe3+ iron) stain. Our results show that the neurological deficit scores in FP-/- mice was significantly higher compared to WT mice (3.1±0.8 vs. 6.1±0.7; $p<0.01$, n=6-10) at 72h after ICH. Also, the total time on the rotarod was significantly less in FP-/- mice than WT mice (27.0±7.5 vs. 52.4±11.2s; $p<0.05$) at 24h after ICH. Using the grip strength to measure forepaw strength after ICH, we show that FP-/- mice had significantly less strength compared to WT mice at 72h after ICH (96.4±17.0 vs.129.6±5.9g; $p<0.01$). In addition to the behavioral outcomes, histopathological measurements were made. From Cresyl violet stained brain sections, FP-/- mice show a significantly greater lesion volume compared WT (15.0±2.2 vs. 3.2±1.7mm3; $p<0.01$). Following GFAP & Iba1 immunoreactivity experiments on brain sections; we show that FP-/- mice have greater Iba1 immunoreactivity than WT mice. To detect and measure the presence of ferric (Fe3+) iron in the peri-hematoma area, Perls' staining was performed and we observe that FP-/- mice have significantly more Perls' stained Fe3+ than compared to WT mice (186.3±34.4% vs 86.9±13.0% total positive counts; $p<0.05$). In conclusion, this study shows that deletion of the FP receptor exacerbates behavioral impairments

following ICH as compared to WT controls. However, the complete mechanism responsible for these novel results is actively being pursued. Currently studies are in progress to measure ICH outcomes following administration of FP receptor drugs.

Disclosures: S. Mohan: None. **E. Koller:** None. **S. Narumiya:** None. **S. Dore:** None.

Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

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Program#/Poster#: 715.05/CC5

Topic: C.21.Stroke Recovery

Support: Department of Biotechnology, Government of India

National Brain Research Centre, Ministry of Science and Technology, Government of India

Title: Modeling of ischemic stroke therapy using endogenous adult neural stem cells - A multi-discplinary patient customised approach

Authors: *S. KAPOOR, V. P. S. RALLABANDI, B. VICTOR, P. K. ROY;
Natl. Brain Res. Ctr., Gurgaon, India

Abstract: The aim is to develop a regenerative therapy design for Transient Ischemic Attack (TIA) using a multi-disciplinary approach. In our study, the phenomena of neurogenesis, synaptogenesis and progenitor cell migration from Sub Ventricual Zone (SVZ), under the influence of therapeutic growth factor introduced interventionally are used as model parameters. Based on a model of gene modulation, we quantified the rate of neural stem cell (NSC) proliferation, migration towards the stroke penumbra, and differentiation into niche specific neurons. This computed information is tested on an animal model of TIA. A predictive mathematical model is designed to discretize the steps of division, migration and differentiation of stem cells leading to neurorestorative recovery, under influence of factors such as IGF-1 and Statins. Upon computing the optimal dosage and time-point of administration, the information is validated in-vivo. A global ischemia model using the Middle Cerebral Arterial Occlusion (MCAO) technique is established. Biochemical analysis is done to assess the potential of therapy. MRI and Diffusion-Perfusion imaging is done to quantify reduction of hypoxic volume post therapy. This model is further verified using systems biology tool MetaDrug which allows

visualization of interactive canonical pathway during pathology and recovery. On analysis of the effect of varying therapeutic doses and time duration of exposure on NSC dynamics, a strong peak of maximal production is observed . The model estimates that the functionally synapsed efficiency is 19% of total matured neurons,. The predicted time of sensorimoror recovery is 3-4 weeks. Also, the calculated speed of neural progenitor cell migration is 45 um/hr. Our simulation results concur with exisitng experimental findings. Preliminary data from in-vivo animal studies reflect similar trends of recovery. The ischemic brain has evolved an incisive way to reorganize itself by increasing the production of endogenous stem cell niches which might integrate into the existing synaptic network as well as provide supporting structure to the ipsilesional and contralesional hemisphere. An approach to augment this performance is by suitably optimizing drug dosage and its temporal scheduling. The strategy has far ranging implications for stroke patients where there is scope for recovery by timely intervention in the penumbra. Our efforts can be seen as the first endeavor of incorporating discrete endogenous NSC processes in a robust model that allows for incorporation of patient specific parameters that would be indicators of recovery which could be monitored effectively by imaging and pathological bio-markers.

Disclosures: **S. Kapoor:** None. **V.P.S. Rallabandi:** None. **B. Victor:** None. **P.K. Roy:** None.

Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 715.06/CC6

Topic: C.21.Stroke Recovery

Title: Why C-fos matters in the bedside treatment of stroke: A new clue in the development of a tolerant brain against ischemic stroke

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Abstract: Since stroke induced ischemia-induced pathobiology have complex and, occasionally, different time courses that can set-in within minutes, hours, and or even days, the development of specific time-dependent therapeutic manipulations becomes a necessity to protect the metabolically vulnerable neurons that are consistently subjected to hypoxia/ischemia insults

following the onset of a stroke. Such specific therapeutic interventions should counter the excitotoxic effects directly linked to the severe restriction of blood flow that causes necrotic cell death - major pathological effect of stroke. The ability to identify the mechanisms that facilitate the thwarting responses would significantly provide a new and innovative therapeutic approach that will deter the onset and downstream pathology of stroke. One of such mechanisms is the role of transcriptions factors such as the immediate early gene during ischemia and tissue hypoxia. In a series of experiments, we observed an increase in c-fos expression in naked mole rats during hypoxia, suggesting that the brain of the naked mole rat is responsive to hypoxia at the level of gene expression involving IEG products, such as c-fos. Our finding suggests that a better understanding of mechanisms that facilitate hypoxia-induced activation of c-fos in a natural system of neuroprotection will inform the development of therapeutics or treatment for a tolerant brain against ischemic stroke.

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Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

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Program#/Poster#: 715.07/CC7

Topic: C.21.Stroke Recovery

Title: Polyethylene glycol modified albumin (PEG-albumin) reduces the infarct size in mouse model of ischemic stroke and cerebral edema

Authors: R. ALGHATANI[1], J. TULSULKAR[2], M. AZIZI[1], V. KAZAN[1], A. R. KHAN[1], M. JUMAA[3], N. ALTOROK[1], J. D. DIGNAM[4], *Z. A. SHAH[5], R. ASSALY[1];
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Abstract: **Background:** Ischemic stroke is the 4th leading cause of death in the United States and a leading cause of significant disability. The cerebral edema complicating large ischemic strokes accounts for the majority of deaths during the first week after the ischemic insult. Apart from neurosurgical interventions, no single agent has been shown to improve mortality and patients' overall outcomes in ischemic strokes complicated by cerebral edema. Therefore, we investigated in this study if Polyethylene Glycol Modified Albumin (PEG-Albumin) could be a

potential therapeutic candidate. **Methods:** In this study, we compare the effect of PEG-Albumin in 3% hypertonic Saline on the infarct size and cerebral edema in mouse model of stroke to either 3% hypertonic saline (HS) or human serum albumin in 3% hypertonic Saline. We used C57Bl/6 mouse strain after performing permanent left middle cerebral artery (MCA) occlusion using electrical cautery. We administered similar volume (based on albumin content at a dose of 0.3g/Kg at a concentration of 120mg/ml) of 3% HS, PEG-Albumin and Albumin after 48 hours of inducing the stroke through intrajugular route. In total, we studied 28 mice 3% HS (n=8), PEG-Albumin in 3% HS (n=10), and Albumin in 3% HS (n=10). Moreover, we assessed the functional outcomes using Neurological Deficit Scale (NDS) designed for mice after 48 hours of administering the three agents. Upon euthanasia, we sliced the brains of the animals and stained it with TriphenylTetrazolium Chloride (TTC) to quantify the infarct size and edema and kept them in formaldehyde for analysis. **Results:** We demonstrate a 40% reduction in the infarct size in mice treated with PEG-Albumin compared to mice treated with 3% HS ($p<0.01$), without statistically significant difference in cerebral edema in mice treated with PEG-Albumin or Albumin compared to 3% HS control group ($p=0.9$, $p=0.7$, respectively). Moreover, there was no statistically significant difference in the infract size between mice treated with PEG-Albumin compared with Albumin HS ($p=0.08$). PEG-Albumin was associated with better functional outcomes on the NDS when compared to Albumin ($p=0.016$). **Conclusion:** This study demonstrates the potential benefit of PEG-Albumin in reducing the infarct size in mouse model of stroke. Moreover, use of PEG-Albumin was associated with less neurological deficits. Further studies are needed to explore the potential benefit of PEG-Albumin in treatment of ischemic stroke.

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Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 715.08/CC8

Topic: C.21.Stroke Recovery

Title: Possible role of Piroxicam in GABA agonism to alleviate Glutamate mediated neuronal insult in rodent model of ischemic stroke

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Abstract: To explore the importance of brain neurotransmitters in the rodent model of focal cerebral ischemia, we evaluated the effects of Piroxicam, a non steroidal anti-inflammatory drug (NSAID) on extracellular brain glutamate and γ-aminobutyric acid (GABA) release, survival time and neuronal cell death. Focal cerebral ischemia led to neuronal infarction and also compromised intrinsic antioxidant status. Thirty minutes pre administration of Piroxicam (10 mg/kg b.w) showed a significant ($P < 0.01$) reduction in cerebral infarct volume and potentiation of the intrinsic antioxidant status as well. Further, high performance liquid chromatography was performed to measure changes in extracellular concentrations of neurotransmitters which were found to be 0.519 ± 0.44 pmole/mg; 1.18 ± 0.28 pmole/mg; 0.63 ± 0.21 pmole/mg respectively while hydroxyl radical (.OH) adduct of salicylate in the frontal cortex and striatum in control, untreated and treated rat models were found to be 0.261 ± 0.06 pmole/mg; 0.68 ± 0.52 pmole/mg; 0.401 ± 0.68 pmole/mg respectively. After the onset of stroke, the extracellular level of glutamate in rat brain increases continuously as compared to that of control group. However, by administration of Piroxicam in stroke rat, the elevated extracellular cerebral glutamate was found to be significantly ($P<0.05$) reduced. This indicates that Piroxicam attenuates extracellular glutamate release and also reduces neuronal cell death due to reduction in oxidative stress in cerebral ischemia. Our results also explain a consequent increase of extracellular GABA in brain regions administered with Piroxicam, which justifies that Piroxicam alleviates glutamate excitotoxicity possibly by GABA agonism.

Disclosures: P. Bhattacharya: None. **A.K. Pandey:** None. **S. Paul:** None. **R. Patnaik:** None.

Poster

715. Stroke Recovery: Rodent Models

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Topic: C.21.Stroke Recovery

Support: NIH Grant NS058710

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Title: Effects of 6-bromoindirubin-3'-oxime on neurogenesis and angiogenesis after hemorrhage stroke in mice

Authors: *Y. ZHAO[1,2], J. LI[1], J. SUN[2], S. YU[2], L. WEI[2,3];
[1]Neurol., Beijing Friendship Hospital,Department of Neurol., Beijing, China; [2]Anesthesiol., Emory Univ., Atlanta, GA; [3]Neurol., Emory Univ., Altanta, GA

Abstract: Intracerebral hemorrhage (ICH) is a devastating disease that accounts for approximately 10% of all strokes, has high mortality rates and results in severe disability in survivors. There are no effective therapies to improve prognosis after ICH. In the present study, we focused on the post-ICH regenerative process in the subventricular zone (SVZ). 6-bromoindirubin-3-oxime (BIO) is a cell-permeable selective inhibitor of glycogen synthase kinase-3 (GSK-3). GSK3 is an important regulator in neurodevelopment and neurogenesis processes, such as neural progenitor proliferation, cell polarization, and migration. We explored the potential promoting effect of BIO on neurogenesis in an ICH model of mice. Adult male C57BL/6 mice (8-10 weeks-old, 25-30g body weight) were randomly divided into sham control, ICH/vehicle control and ICH/BIO treatment groups. ICH injury was induced by Collagenase IV subcerebral injection. BIO or vehicle treatment was started 3 days after ICH and repeated every 2 days until the day before sacrifice. BIO enhanced cell proliferation in the SVZ, neuroblast migration towards the lesion region, and increased angiogenic activity in the peri-hematoma region 14 days after ICH. In functional assays, the Neurological Severity Score (NSS) and Rotarod test showed remarkable functional recovery in ICH mice that received BIO treatment. We concluded that BIO can be a promising treatment for increased brain regeneration and functional recovery after ICH.

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Poster

715. Stroke Recovery: Rodent Models

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Topic: C.21.Stroke Recovery

Support: NIH grant R01 NS060911

Title: Stem cell factor and granulocyte colony-stimulating factor regulate neurite extension through p53

Authors: *L.-R. ZHAO, M. GAO;
SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Stem cell factor and granulocyte colony-stimulating factor regulate neurite extension through p53 Mei Gao and Li-Ru Zhao Department of Neurosurgery, State University of New York, Upstate Medical University, Syracuse, New York 13210, USA. Stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) are the essential hematopoietic growth factors to regulate hematopoietic stem cell survival, growth and differentiation. Accumulating evidence suggests that SCF and G-CSF may also contribute to neuronal plasticity. We have recently demonstrated that SCF in combination with G-CSF (SCF+G-CSF) increases axonal sprouting, dendritic branching, and synaptogenesis in the brain of chronic stroke. However, the mechanism underlying the SCF+G-CSF-induced neuronal network reorganization remains poorly understood. Neurite outgrowth is the initial process for neurons to build the neuronal networks. Here we have determined the involvement of MEK/ERK/p53 signaling in SCF+G-CSF-regulated neurite outgrowth using primary neuronal cultures. We observed that SCF+G-CSF promotes neurite extension through activating MEK/ERK signaling. Remarkably, p53, a tumor suppressor protein, was significantly increased by SCF+G-CSF via MEK/ERK pathway. Knocking down p53 by p53siRNAs leads to prevention of the SCF+G-CSF-induced enhancement of neurite outgrowth. These data suggest that MEK/ERK/p53 signaling is required for SCF+G-CSF-promoted neurite outgrowth. This study demonstrates a novel role for p53, which is distinct from its pro-apoptotic effects, on supporting neurite extension by SCF+G-CSF treatment. This study was supported by The National Institutes of Health, National Institute of Neurological Disorders and Stroke (NINDS), R01 NS060911.

Disclosures: L. Zhao: None. **M. Gao:** None.

Poster

715. Stroke Recovery: Rodent Models

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Topic: C.21.Stroke Recovery

Support: JSPS KAKENHI Grant Number 24800054

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Title: Contribution of the cortico-rubral axons to recovery by the constraint-induced movement therapy in capsular hemorrhage rats

Authors: *A. ISHIDA[1], K. ISA[2], K. KOBAYASHI[3], T. UMEDA[2], T. ISA[2], H. HIDA[1]; [1]Mizuhocho, Nagoya City Univ. Grad. Sch. of Med. Sci., Nagoya, Aichi, Japan; [2]Dept. Dev. Physiol., Natl. Inst. Physiol. Sci., Okazaki, Japan; [3]Div. Viral Vector Dev., Natl, Inst. Physiol. Sci., Okazaki, Japan

Abstract: Constraint-induced movement therapy (CIMT) has been known as an effective treatment promoting motor recovery of upper extremity function after stroke. However, the detailed reorganization of CNS induced by CIMT remained unknown. The aim of the present study was to clarify the CIMT-induced circuit-level reconstruction and the causal connection with functional recovery after intracerebral hemorrhage (ICH) using double-infection technique for pathway-selective blockade. Adult Wistar rats were divided into sham-operated group, ICH group, and ICH-CIMT group. Collagenase (type IV, 15 Units/ml, 1.4 µl) was injected into internal capsule of ICH group rats. On the postoperative days 1-8, ICH-CIMT group rats were fitted a one-sleeve cast to perform all the daily movements only with their affected limb. Behavioral assessments (skilled reaching and ladder stepping) showed better recovery in ICH-CIMT group than in non-treated control group. Serial mapping of the ipsilesional motor cortex by intracortical microstimulation demonstrated the larger forelimb representative area in the ICH-CIMT group compared to ICH group on the postoperative days 10 and 26. Microinjection of muscimol (1 µM, 1 µl) into the founded forelimb areas by ICMS decreased the behavioral performance. Anterograde tracing by biotinylated dextran amine (BDA) injection into the areas (each 0.5 µl, 2 sites) demonstrated the abundant axonal projections from the motor cortex forelimb area to the red nucleus in ICH-CIMT group. Finally, we blocked the cortico-rubral pathway selectively by a double-infection technique (Kinoshita et al., 2012, Sooksawate et al. 2013). A retrograde gene transfer vector with the gene encoding enhanced tetanus neurotoxin under the control of a tetracycline responsive element was injected into the contralesional red nucleus and subsequent injection of adeno-associated viral vector with a highly efficient Tet-ON sequence at the ipsilesional motor cortex. After 6 weeks, impairment of skilled reaching task of ICH-CIMT group was found when the administration of doxycycline (DOX) was initiated. DOX-treated CIMT rats showed apparent deficit of limb extension to reach for the pellets. These data suggested that the CIMT promoted reorganization of the ipsilesional motor cortex and enhanced the connection of cortico-rubral pathway as a substrate for functional recovery.

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Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

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Program#/Poster#: 715.12/CC12

Topic: C.21.Stroke Recovery

Title: Impact of cerebral inflammation and mechanical disruption operation on neovascularization in mild ischemic rat model

Authors: ***G. PARK**[1], K.-E. LEE[2], J. LEE[2], J. HONG[2];
[1]Dept. of biomedical science, Ajou Univ. Sch. of Med., Suwon, Korea, Republic of; [2]Dept. of Neurol., Ajou university Sch. of Med., Suwon, Korea, Republic of

Abstract: Background: Neovascularization is a promising restoration strategy for the acute stroke. Cerebral inflammation and disruptions of blood brain barrier are essential for the development of neovascular networks in mild and severe ischemic models. Method: Our experiment focused on mild ischemic stroke model. Both hemispheric hypoperfusions were induced by internal carotid artery ligations. Cerebral inflammation was systemically produced with lipopolysaccharide (LPS, 0.1mg/kg) for 3 days after ligations and mechanically barrier disruption was performed as 3 mm burr-hole operation on the right side. In comparison of both hemispheric blood flows, laser Doppler flowmetry was used. Neovascularization from extracranial portion was grossly compared with Evans-blue dye injection (1.5 cc) to external carotid artery at 14 days after surgery. Vessel density, angiogenic factors, and inflammation markers were evaluated by immunohistochemical analysis at 14 days after surgery. Results: In comparison of cerebral inflammation (CI) group (n=5), mechanical disruption (MD) group (n=5), and saline control (n=5), combination of both treatments (CI + MD) group (n=5) showed significant elevations of vessel density ($p<0.05$), angiogenic factors including vascular endothelial growth factor (VEGF) ($p<0.05$), and inflammatory markers ($p<0.05$) on an adjacent burr-hole. MD group also showed a little neovascularization findings. However, there is no neovascualization evidence in CI and saline control groups. Conclusions: Our findings suggest that the combination of cerebral inflammation and mechanical disruption operation be crucial to neovascularization in mild ischemic rat model.

Disclosures: **G. Park:** None. **K. Lee:** None. **J. Lee:** None. **J. Hong:** None.

Poster

715. Stroke Recovery: Rodent Models

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Title: Excitotoxic insult results in a persistent activation of CaMKIIα in living hippocampal neurons and is linked to mitochondrial fragmentation

Authors: *N. OTMAKHOV[1], E. GORBACHEVA[1], S. REGMI[1], R. YASUDA[2], A. HUDMON[3], J. LISMAN[1];
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Abstract: Over-activation of excitatory NMDA receptors and the resulting Ca2+ overload is the main cause of neuronal toxicity during stroke. CaMKII becomes misregulated during such events. Biochemical studies show either a dramatic loss of CaMKII activity or its persistent autonomous activation after stroke, with both of these processes being implicated in cell toxicity. To complement the biochemical data, we monitored CaMKII activation in living hippocampal neurons in slice cultures using high spatial/temporal resolution two-photon imaging of the CaMKIIα FRET sensor, Camui. CaMKII activity was estimated by measuring Camui fluorescence lifetime. Short NMDA insult resulted in Camui activation followed by an increase of Camui content in spines a decrease in dendritic shafts, and redistribution to numerous clusters in the cell soma. Camui activation was either persistent (> 3 hours) or transient (~20 min) and, in general, correlated with its content redistribution. In a large group of cells, however, the content redistribution persisted longer than Camui activation, suggesting distinct regulation/phases of these processes. Mutational and pharmacological analysis suggested that persistent Camui activation was due to prolonged Ca2+ elevation, with little impact of autonomous states produced by T286 autophosphorylation and/or by C280/M281 oxidation. Shortly after Camui activation and clustering, NMDA treatment resulted in mitochondrial fragmentation, with persistence of the fragmentation linked to the persistence of Camui activation. CaMK or

calmodulin inhibitors (KN93 or W7) suppressed CaMKII activation and dramatically decreased mitochondrial fragmentation. The results suggest that excitotoxic insults can produce persistent Ca2+-dependent active conformation of CaMKII, with catalytic activity restricted due to clustering. In addition, they point to a new mechanism for the involvement of CaMKII in cell damage through mitochondrial fragmentation.

Disclosures: N. Otmakhov: None. **E. Gorbacheva:** None. **S. Regmi:** None. **J. Lisman:** None. **R. Yasuda:** None. **A. Hudmon:** None.

Poster

715. Stroke Recovery: Rodent Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 715.14/CC15

Topic: C.21.Stroke Recovery

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NIH grant NS058710

NIH grant NS062097

NIH grant NS075338

AHA 0840110N

Title: Characteristics of delayed neuronal cell death in the ischemic core following focal cerebral ischemia in mice

Authors: *M. Q. JIANG[1], Y. ZHAO[2,4], X. GU[2], L. WEI[2,3], S. YU[2];
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Abstract: Ischemic stroke is the third most prevalent cause of death and the leading cause of long term disability with direct and indirect costs amounting to 36.5 billion annually in the United States. Studies of ischemic stroke have focused on evaluating the ischemic penumbra as a target for therapeutic intervention, however we have recently demonstrated significant levels of viable neurons in the ischemic core up to 7 days following ischemic stroke. Neuronal and non-neuronal cells resistant to acute ischemic damage in the infarct core represent a small but

significant population of cells which may provide an overlooked cell population for tissue repair and endogenous support for cell transplantation therapies. In this study we evaluate the characteristics of delayed neuronal cell death in the ischemia core via TUNEL staining and compared this population of neurons to those neurons which undergo either acute cell death or undergo cell death in the penumbra. Adult C57/B6 male mice were subjected to permanent distal MCA occlusion with 7 min CCA occlusion. Mice were sacrificed and histological examinations were performed on 10-μm sections at time points of 6, 12, 24, 48, 72 hrs, 7 days, and 14 days after stroke. Cell death and proliferation were quantified using terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and 5-bromo-2'-deoxyuridine (BrdU). We delineated the ischemic core from the ischemic penumbra by quantifying robust reactive astroglial activation forming a scar around the infarcted area. Reactive astrocytes stain positive for GFAP at 6 hours following ischemia and the number of reactive astrocyte processes increases significantly by 7 days. A glial scar border marked by GFAP staining appeared by 48 hours following ischemia. A small but noticeable population of neurons survived for at least 7 days after stroke in the ischemic core. Delayed neuronal cell death within the ischemic core at 7-14 days was compared to acute neuronal cell death and neuronal cell death in the penumbra. Neurons which stain positive for TUNEL in the ischemic core are significantly larger with a mean cross section area of 100.9 ± 3.65 um, compared to 55.04 ± 1.59 um ($n=3$, $p<0.001$) in the penumbra region. TUNEL type I neuronal cell death were found more during acute cell death at 24 hours following ischemia or in the penumbra at delayed time points. Furthermore, significantly more neurons in the core are categorized under TUNEL type II cell death featuring fragmented nuclei and membrane deterioration indicative of a mixed form of hybrid cell death. These observations provide new information on the pathogenic processes in the ischemic core and penumbra regions.

Disclosures: **M.Q. Jiang:** None. **Y. Zhao:** None. **X. Gu:** None. **L. Wei:** None. **S. Yu:** None.

Poster

715. Stroke Recovery: Rodent Models

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The 973 program of China (2011CB510004)

Key Project Grant of NSFC (31330035)

HKGRF Grant (466713)

Title: Early post-stroke administration of L-DOPA dramatically improves motor function recovery in rats

Authors: *L. YAN, Q. LI, W. H. YUNG, Y. KE;
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Abstract: Compromised blood flow to the brain during a stroke can cause permanent neuronal loss and dysfunction of the brain tissue. Recent studies have shown that L-DOPA, a drug which is used in the clinical treatment of Parkinson's disease via restoring central dopamine level, can also substantially improve motor performance in stroke patients. In our study, we tested the hypothesis that L-DOPA acts on the motor cortex to enhance functional recovery. Two-months old Sprague Dawley rats received vehicle or L-DOPA 15 mg/kg daily (i.p.) for 3 weeks following ischemic stroke induced via unilateral stereotaxic injection of endothelin-1 into the cortex. The rats underwent motor behavior evaluation by means of open field test, rotarod performance test, horizontal ladder test and limb use asymmetry assessment. Cresyl violet, triphenyltetrazolium chloride and immunohistological staining of tyrosine-hydroxylase (TH) were performed to compare the difference in stroke volume and the integrity of dopaminergic projection in the motor cortex of L-DOPA-treated and control animals both pre- and post-ischemic injury. Stroke volume analysis demonstrates infarct area was confined to the cortex and infarct core was centered on M1 in our model. TH-positive terminals were markedly reduced in the affected hemisphere. When compared with control, L-DOPA-treated animals displayed fewer errors in horizontal ladder test throughout all testing sessions post-stroke. At day20, L-DOPA group displayed a mean reduction of error in both forelimb and hindlimb (13.5% and 11.6% respectively). The L-DOPA-treated animals also exhibited improved performance in rotarod test, spending an average of 69.5±6.3s longer on the rod than vehicle group in each testing session post-stroke, indicating an improved limb placing ability, balance and inter-limb coordination. In addition, stroke animals exhibited significant asymmetric limb use which persisted throughout the testing period post-stroke. However, L-DOPA-treated animals showed partial restoration of symmetric limb-use progressively. Cortical DA system is significantly disrupted by focal stroke of the cortex. A 3-week daily treatment of L-DOPA can substantially improve motor performance in rat model of cortical stroke, suggesting that dopamine replacement can enhance functional recovery and that restoring cortical DA level is essential for post-stroke rehabilitation.

Disclosures: **L. Yan:** None. **Q. Li:** None. **W.H. Yung:** None. **Y. Ke:** None.

Poster

715. Stroke Recovery: Rodent Models

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Program#/Poster#: 715.16/CC16

Topic: C.21.Stroke Recovery

Title: A novel automated method for isolating and quantifying supination performance in a rat model of ischemic stroke

Authors: *E. MEYERS[1], A. SINDHURAKAR[3], S. HAYS[1], A. SLOAN[1], J. CARMEL[3,4], M. KILGARD[2,1], R. RENNAKER[1,2];
[1]Erik Jonsson Sch. of Engin. and Computer Sci., [2]Sch. of Behavioral Brain Sci., Univ. of Texas At Dallas, Richardson, TX; [3]Motor Recovery Lab., Burke Med. Res. Inst., White Plains, NY; [4]Brain and Mind Res. Inst. and Departments of Neurol. and Pediatrics, Weill Med. Col. of Cornell Univ., New York, NY

Abstract: Stroke affects millions each year, often resulting in loss of motor function. One common manifestation of motor dysfunction is impairment of forelimb musculature controlling pronation and supination. Given the prevalence of deficits in forelimb rotation, it would be valuable to accurately model the dysfunction in rodents. The current gold standard tasks of measuring forearm rotation in rodents are prone to human error, problems in different scoring systems, laborious, and lack the sensitivity to detect subtle motor deficits that manifest themselves in supination. Here we describe a novel automated, high-throughput method to quantitatively measure deficits in forelimb rotation in a rat model of ischemic stroke. The task requires animals to reach through a small aperture in a clear acrylic cage, grasp a spherical knob, and then supinate to receive a reward pellet. A rotary encoder tracks the angular position of the knob with a resolution of one-quarter of a degree. With a rich dataset of ~300 trials a day per animal, we can quantitatively analyze each rat's performance with minimal human bias. Once the animal is trained to the specified degree threshold, a unilateral primary motor cortex ischemic lesion (Endothelin-1) is administered and the animal's performance is then followed for 4 weeks post lesion. Adult female Sprague-Dawley rats were used and all handling, housing, surgical procedures, and behavioral training of the rats were approved by the University of Texas Institutional Animal Care and Use Committee. After training to proficiency of 80% success rate for two consecutive days with a 60-degree threshold, animals exhibit a chronic supination deficit following ischemic motor cortex damage compared to pre-lesion performance. Measures of performance, including mean distance turned and rate of success show severe and long-lasting

impairments even with intensive rehabilitation of ~300 trials per day. Mean performance of five animals dropped from 66 degrees pre-lesion to 34 degrees after four weeks of intensive rehabilitation. We have developed a novel supination assessment task that provides accurate, high-resolution quantitative assessment of distal forearm rotation in rodents. Our preliminary results indicate that the task can detect chronic deficits in animals with an ischemic stroke. Future experiments will assess the ability of the task to detect deficits in forelimb rotation in models of other neurological injuries including spinal cord injury, traumatic brain injury, and Parkinson's disease. Using this task we will assess paired vagus nerve stimulation (VNS) as a potential therapy for restoring rotational motion following ischemic stroke.

Disclosures: E. Meyers: None. **A. Sindhurakar:** None. **S. Hays:** None. **A. Sloan:** None. **J. Carmel:** None. **M. Kilgard:** None. **R. Rennaker:** None.

Poster

715. Stroke Recovery: Rodent Models

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 715.17/CC17

Topic: C.21.Stroke Recovery

Title: The effect of inducible nitric oxide synthase inhibition on development of local hyperthermia-induced morpho-physiological changes in the brain tissue of rats

Authors: *L. GOBECHIA-DAVLIANIDZE, M. DEVDARIANI, N. MOMTSELIDZE, M. MANTSKAVA, M. NEBIERIDZE, N. MITAGVARIA;
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Abstract: It is known that hyperthermia stimulates a sharp increase in expression of Nitric Oxide Synthases (NOS). Despite the fact that the basal endothelial Nitric Oxide (NO) plays an important protective role - prevents platelet aggregation and constriction of blood vessels, NO produced by activation of inducible Nitric Oxide Synthase (iNOS) reaches such proportions that formed peroxynitrite causes platelets aggregation, induction of vascular hyperreactivity, aggravation of blood rheological properties, etc. All these cause thrombosis of blood vessels and decrease of tissue thermoclearance. A vicious circle develops with a complete breakdown of microcirculation and necrosis in the appropriate tissue site. In case of tumor tissue these are positive events, but hyperthermia also acts on normal, adjacent tissue, in which such processes are detrimental. The aim of this study was evaluation of iNOS inhibition effect on development of described processes in normal brain tissue. Experiments were performed on rats, anesthetized

by Chloral Hydrate solution. The skull was exposed and dura mater retracted. The local hyperthermia was induced by irrigation of brain surface with heated up to 430C saline in both experimental and the control groups. We revealed that inhibition of iNOS by i/p administration of Aminoguanidine in experimental group significantly reduced (in comparison with control one) the number of thrombosed cortical vessels, the local blood flow and tissue oxygenation was maintained an appropriate levels. Morphological examination of hyperthermia exposed brain tissue did not revealed all those significant damages that were observed in control group of animals.

Disclosures: L. Gobechia-Davlianidze: None. **M. Devdariani:** None. **N. Momtselidze:** None. **M. Mantskava:** None. **M. Nebieridze:** None. **N. Mitagvaria:** None.

Poster

716. Stroke Recovery

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Topic: C.21.Stroke Recovery

Support: EU Grant FP7 Collaborative Projects ARISE(201024) and PLASTICISE(223524)

ERC grant NOGORISE

Swiss National Foundation grants Nr. 31-138676 and 3100A0_12252711

Title: Restoration and manipulation of regained forelimb function after stroke

Authors: *A.-S. E. WAHL[1], W. OMLOR[2], B. ANTIC[3], S. MUSALL[2], J. C. RUBIO[3], A. SCHRÖTER[4], M. GULLO[1], H. KASPER[1], O. WEINMANN[1], F. HELMCHEN[2], B. OMMER[3], M. E. SCHWAB[1];
[1]Brain Res. Institute, Univ. and ETH Zurich, Zuerich, Switzerland; [2]Brain Res. Institute,University of Zurich, Zurich, Switzerland; [3]Computer Vision Group, Heidelberg Collaboratory for Image Processing and IWR, Univ. of Heidelberg, Heidelberg, Germany; [4]Inst. for Biomed. Engineering, ETH Zurich, Zurich, Switzerland

Abstract: The adult nervous system reveals limited capacities by which neural networks can reorganize and reassemble in modified configuration to sustain behavioural recovery and compensation after stroke. Current strategies to improve long-term outcome include mostly rehabilitative training and in experimental models electrical stimulation and pharmacological

interventions. However, the scientific basis for designing rehabilitative schedules is poorly understood. Here we show almost full recovery of skilled forelimb functions in rats with large photothrombotic strokes of the sensorimotor cortex when a growth promoting immunotherapy against a neurite growth inhibitory protein (Nogo-A) was applied to boost the sprouting of new fibers, followed by 2 weeks of intensive reaching training to select, stabilize and prune the newly formed circuits.. Anatomically, anti-Nogo-A antibodies induced large numbers of corticospinal fibers from the intact side to grow across the midline of the cervical spinal cord. Using three different pharmaco- and optogenetic approaches we temporalily silenced the corticospinal fibers that have grown from the intact motor cortex into the ipsilateral, stroke-denervated hemicord: the very well restored skilled reaching of the stroke-affected forelimb was fully abolished by silencing these neurons, showing the functional importance of the newly formed ipsilateral corticospinal projection.

Disclosures: **A.E. Wahl:** None. **W. Omlor:** None. **B. Antic:** None. **S. Musall:** None. **J.C. Rubio:** None. **A. Schröter:** None. **M. Gullo:** None. **H. Kasper:** None. **O. Weinmann:** None. **F. Helmchen:** None. **B. Ommer:** None. **M.E. Schwab:** None.

Poster

716. Stroke Recovery

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 716.02/CC19

Topic: C.21.Stroke Recovery

Title: Cervical trans-spinal direct current stimulation activates dormant connections after pyramidal tract injury

Authors: A. AMER, *Z. AHMED;
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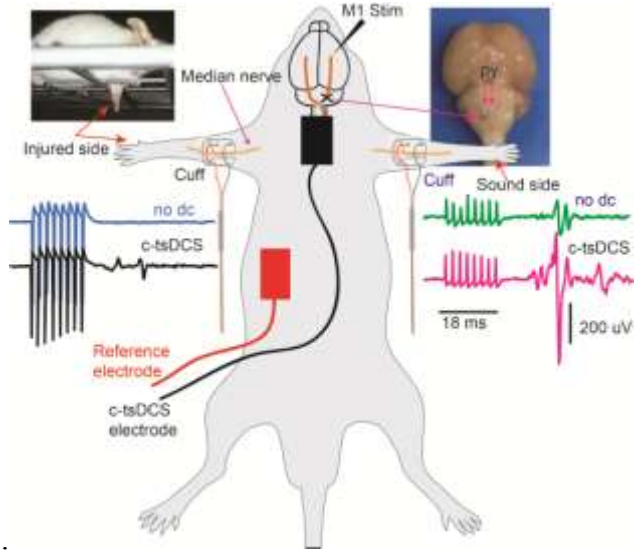
Abstract: Cathodal trans-spinal direct current stimulation (c-tsDCS) enhances the excitability of spinal neurons. This can be used to activate and strengthen dormant neural pathway/s to compensate for injured primary pathways. To test for this idea, here we unilaterally transected the pyramidal tract (“py.”). In response to this injury, mice showed significant impairment in the way they used their fore- and hindlimbs contralateral to the injured corticospinal tract (e.g. step slippage; see figure). In acute experiments, cortically evoked median nerve responses were potentiated during and after cervical cathodal tsDCS in healthy animals. In animals with pyramidotomy (“X”), stimulation of the ipsilesional motor cortex (M1) evoked no response from

the contralesional median nerve (no dc, blue trace in the figure) however, a response was evoked during c-tsDCS (black trace in the figure). Moreover, ipsilesional M1 stimulation evoked a response in ipsilesional median nerve (sound side; green trace), which was enhanced during c-tsDCS (magenta trace). These responses were potentiated by brief (30 second) combined c-tsDCS with high frequency cortical stimulation. Potentiation was persistent for at least 30 minutes beyond the stimulation period. In addition, stimulation of the uninjured corticospinal tract evoked bilateral median nerve responses, which were also potentiated by c-tsDCS. These observations indicate that cervical cathodal tsDCS can be used to activate/potentiate compensatory pathways following brain injury (e.g. stroke). These indirect cortex to spinal cord pathways are most likely through cortico-rubrospinal or/and cortico-reticulospinal routes.



Disclosures: **A. Amer:** None. **Z. Ahmed:** None.

Poster

716. Stroke Recovery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 716.03/CC20

Topic: C.21.Stroke Recovery

Title: Effects of cathodal transcranial direct current stimulation with robotic therapy on arm function in subacute stroke patients

Authors: *M. SOHN, S. JEE, J. LIM;
Rehabil. Med., Chungnam Natl. Univ. Hosp., Daejeon, Korea, Republic of

Abstract: Objective: To examine the effects of combined therapy using transcranial direct current stimulation (tDCS) with robot-assisted arm training (AT) for impairment of the upper limb in subacute hemiplegic stroke patients Methods: 20 subacute first-ever hemiplegic stroke patients with moderate-to-severe arm paresis t randomized 2 groups: group A received cathodal stimulation of the nonlesioned hemisphere, for 20 minutes at 1.0 mA, and group B received sham stimulation for 2 weeks. The electrodes were placed over the motor cortex representation of biceps brachii muscle and above the contralateral supraorbital area. Contemporaneously, the subjects practiced 2 sets of 320 repetitions using a shoulder/arm robotic manipulandum (InteractiveMotion Technologies, MA, USA). Outcomes were identified as changes in Fugl-Meyer assessment for the upper limb (FM-UL), modified Ashworth scale (MAS) of shoulder and elbow joint and Korean version of modified Barthel index (K-MBI) at before, after 1 week and 2 weeks intervention. Result: Baseline scores for FM-UL, MAS and K-MBI were comparable in two groups. The FM-UL and K-MBI score improved in both group at 1 and 2 weeks ($P < 0.05$). The FM-UL score was significantly improved in cathodal tDCS group at 2 week. No between-group differences were found in MAS and K-MBI. No major side effects occurred. Conclusion: Cathodal transcranial direct current stimulation enhanced the effect of unilateral arm training in this exploratory trial of subacute stroke patients. Further studies with larger sample size need to be conducted to validate our results.

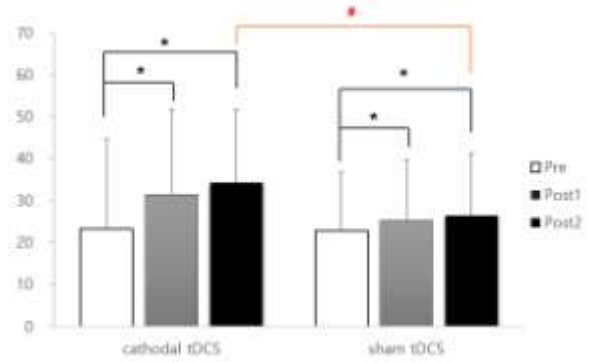


Fig. 1. FM-UL score improved in both group at 1 and 2 weeks (*P < 0.05, ANOVA). The FM-UL score was significantly improved in cathodal tDCS group at 2 weeks(*P <0.05, Mann-Whitney U test).

Disclosures: **M. Sohn:** None. **S. Jee:** None. **J. Lim:** None.

Poster

716. Stroke Recovery

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Topic: C.21.Stroke Recovery

Support: Department of Veteran Affairs Office of Academic Affiliations

RR&D VA

Doris Duke Charitable Foundation

Title: The calibration and limitations of a low cost data glove

Authors: *J. M. GODLOVE[1], A. TSU[2], K. GANGULY[1,2];
[1]Univ. California San Francisco, San Francisco, CA; [2]San Francisco VA Med. Ctr., San Francisco, CA

Abstract: Introduction: Stroke is a leading cause of motor disability in the world. Impaired hand function after stroke is an important impediment to overall functional gains with recovery of the upper limb. Therapies for the upper limb that do not improve fine motor control of the fingers may also have limited impact on disability and quality of life in the modern era. Thus, there is increasing clinical interest in the rehabilitation of hand and finger function after stroke. Background: Data glove technology has been historically manufactured for motion capture and virtual reality applications. A low-cost data glove system appeals to the clinical arena as a potential evaluation tool of hand/finger movement impairments as well as a rehabilitation tool for the restoration of distal function. Although there have been numerous studies across various clinical applications, the calibration procedure for data gloves have not been fully described in these studies. In order to facilitate consistent methodology for the study of this promising clinical device, we report our laboratory's experience with the systematic calibration of a data glove. Methods: The 5-DT Data Glove was chosen because of its low cost and commercial availability. It utilizes bend-sensitive resistors positioned to capture and quantify joint angular displacements. The sensitivity of the resistors itself are dependent on the relative amount of displacement and can vary from angle to angle, thus making straightforward linear calibration unreliable. We established a calibration and regression system that takes into account the non-linear nature of the glove in order to accurately measure finger movement angles (degrees moved). Results: We established a brief protocol to calibrate the system to more reliably measure finger movement kinematics. Using this protocol, we were able to measure finger movements within +/-7° within an optimal functional range of 30-70° of finger flexion. Movements outside of this range were still detected but were found to be less accurate and with a greater amount of uncertainty. We used MATLAB to develop a real-time interface to measure finger position in real-time. We anticipate that this environment will be a power platform for hand and finger rehabilitation studies. Conclusion: We have outlined a simple MATLAB based method for calibrating a low

cost resistor-based data glove. Significant limitations in the linearity and resolution were illuminated using this basic device calibration method. Despite these limitations, we were able to establish a protocol for accurate measurements as well as the development of a real-time monitoring environment for rehabilitation studies.

Disclosures: J.M. Godlove: None. **A. Tsu:** None. **K. Ganguly:** None.

Poster

716. Stroke Recovery

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Support: Minnesota Medical Foundation

National Center for Research Resources to the University of Minnesota CTSI (1UL1RR033183)

Promotion of Doctoral Studies Fellowship, Foundation for Physical Therapy

Title: 6-Hz primed vs. unprimed low-frequency repetitive transcranial magnetic stimulation in stroke

Authors: *J. M. CASSIDY[1], D. C. ANDERSON[2], M. CHEN[1], L. SNOW[1], W. THOMAS[3], J. R. CAREY[1];
[1]Dept. of Physical Med. and Rehabil., [2]Dept. of Neurology, Minnesota Med. Sch., [3]Div. of Biostatistics, Sch. of Publ. Hlth., Univ. of Minnesota, Minneapolis, MN

Abstract: *Objective:* Stroke not only results in the destruction of neural tissue but an alteration in brain hemisphere excitability that further impedes motor recovery. An imbalance of transcallosally-mediated interhemispheric inhibition (IHI) can occur whereby the contralesional primary motor cortex (M1) inhibits ipsilesional M1 to a greater degree than the reverse direction. Repetitive transcranial magnetic stimulation (rTMS) can condition surviving but idle neurons in ipsilesional M1 to enhance their probability of activation during voluntary movement of the affected extremity. Application of suppressive, low-frequency rTMS to contralesional M1 can enhance excitability in ipsilesional M1. It has been shown in healthy individuals that preceding low-frequency rTMS with a train of excitatory, high-frequency stimulation, referred to as priming, can potentiate the effects of the low-frequency stimulation. The objective of this

ongoing study is to compare changes in brain excitability following three types of primed rTMS to ascertain if similar homeostatic-like mechanisms of plasticity translate to the stroke brain. *Methods:* Thus far, using a single-blind crossover design with 1-week washout, 7 adults with chronic stroke received all three treatments to contralesional M1 in randomized order: 1) 10 minutes of active high-frequency priming + 10 minutes of active low-frequency rTMS, 2) 10 minutes of sham high-frequency priming + 10 minutes of active low-frequency rTMS, and 3) 10 minutes of active low-frequency priming + 10 minutes of active low-frequency rTMS. Treatment occurs on Wednesday with pretests on Monday and Tuesday and posttests occurring immediately after treatment and on Thursday and Friday. Measures of cortical excitability include unilateral and bilateral paired-pulse and cortical silent period TMS testing. We compared rTMS treatments using a mixed-effects linear model for change from mean of pretests at each posttest separately with participant as the random effect to model within-subject correlation of repeated measurements. *Results:* Treatments are coded as A, B or C. The code identifying the actual treatment has not been broken yet as the study is in progress until August, 2014. The decoded results will be reported at the November, 2014 SFN meeting. Significant change in bilateral paired-pulse testing occurred with reduced contralesional-to-ipsilesional IHI following Treatment A on posttest 1 (p=0.01) and 2 (p=0.01). Treatment A was superior to B on posttest 1 (p=0.04) and superior to C (p=0.04) on posttest 2. *Conclusion:* Preliminary results suggest that Treatment A is superior to Treatments B and C in reducing IHI from contralesional M1.

Disclosures: **J.M. Cassidy:** A. Employment/Salary (full or part-time):; Gillette Children's Specialty Healthcare. **D.C. Anderson:** A. Employment/Salary (full or part-time):; Hennepin County Medical Center. **M. Chen:** None. **L. Snow:** A. Employment/Salary (full or part-time):; Minneapolis VA Healthcare System. **W. Thomas:** None. **J.R. Carey:** None.

Poster

716. Stroke Recovery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 716.06/CC23

Topic: C.21.Stroke Recovery

Support: Florida Biomedical Research Foundation 3KN01

Title: Error augmentation in chronic stroke: Behavioral & neuroanatomical correlates

Authors: *D. B. ARCHER, G. MISRA, D. E. VAILLANCOURT, C. PATTEN, S. A. COOMBES;
Univ. of Florida, Gainesville, FL

Abstract: It is well documented that a stroke causes changes in brain structure and motor function, and that motor deficits are dependent on the location and size of the infarct. What is less known is if stroke-related motor deficits can be attenuated using augmented visual feedback, and if it is neuroanatomically based. Magnifying error has been shown to improve accuracy and reduce variability during a force production task in healthy adults, but it is not clear if these findings translate to persons post stroke. It has also been demonstrated that microstructural properties of the posterior thalamic radiation (PTR) is correlated with dynamic visuomotor task performance in traumatic brain injury, consistent with the idea that the PTR is critical for visually guided motor tasks. Here, we manipulate the gain of a visual display to examine the effects of error augmentation on unimanual grip force performance in chronic stroke subjects and age- and gender- matched controls. In addition to analyzing force performance, we collected 64 direction diffusion weighted imaging data to quantify brain microstructure and to identify correlations between brain structure and force performance. Our findings show that both the stroke group and the control group significantly attenuated variability and increased accuracy on both hands as gain increased. Comparing the impaired hand of the stroke group to the non-dominant hand of control group, the stroke group had significantly increased variability and decreased accuracy. In contrast, there were no differences when comparing the unimpaired hand of the stroke group to the dominant hand of the control group. Significant negative correlations were found in the PTR between FA and force variability at the high gain level for the impaired/non-dominant hand. FA in the PTR was also significantly reduced in the stroke group as compared to the control group. Probabilistic tractography, using the PTR as a seed, identified tracts that linked the PTR to the superior parietal lobe, thalamus, and inferior temporal gyrus. FA in these tracts was also significantly reduced in the stroke group compared to the control group. In conclusion, error augmentation is a viable method by which to attenuate force variability and increase force accuracy after stroke, but an individual's ability to benefit from error augmented is related to the microstructural properties of the PTR.

Disclosures: D.B. Archer: None. **G. Misra:** None. **D.E. Vaillancourt:** None. **C. Patten:** None. **S.A. Coombes:** None.

Poster

716. Stroke Recovery

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Program#/Poster#: 716.07/CC24

Topic: C.21.Stroke Recovery

Support: Alberta Innovates - Health Solutions

Project SMART

Title: Prevention of deep vein thrombosis using intermittent electrical stimulation

Authors: *E. N. RAVID[1], S. GRAY[2], V. K. MUSHAHWAR[3];
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Abstract: Deep vein thrombosis (DVT) refers to blood clots that form in deep veins, most often in the legs. A life threatening pulmonary embolism (PE) develops if a clot dislodges and reaches the lungs. DVT is fast becoming a health care priority in the United States. In 2008, the US Surgeon General published a call to action to prevent DVT and PE, which together cause 100,000-180,000 deaths/year. Moreover, within two years of an initial DVT event, 23-60% of individuals develop post thrombotic syndrome (PTS) (Ashrani et al. 2009). This is a chronic condition with symptoms that include persistent pain, edema and in severe cases, painful venous ulcers. DVT and PTS create a heavy burden on the health care system and on the work place (Ashrani et al. 2009). Risk factors for DVT are those that promote venous stasis or hyper-coagulable states such as immobility, surgery, pregnancy, or traumatic brain, spinal cord and skeletal injury. Preventive strategies include injections of anticoagulants (e.g. heparin) to reduce coagulability or simulation of the muscle pump action of the lower limbs using intermittent pneumatic compression (IPC) to reduce stasis. IPC was recently shown to be effective in some populations (Dennis et al. 2013); however, low patient compliance and cumbersome use hinders its clinical utility (Camerota et al. 1996). Electrical stimulation (ES) of the muscle pump may be a potential means for preventing DVT. ES delivered to the calf muscle has already been shown to improve blood flow in the leg (Broderick et al. 2010), suggesting it may help to reduce stasis. In this study, the use of intermittent ES to prevent DVT will be explored. Intermittent ES consists of a short “on” time followed by a longer “off” time. Two study groups will be examined: 1) healthy adults and 2) individuals at risk of DVT. ES will be applied to the calf muscles and the hemodynamic response in the popliteal vein will be evaluated. Various stimulation parameters will be tested. Exploratory investigations indicated that 1 s of tetanic stimulation of the gastrocnemius muscle is sufficient to increase blood flow 4 fold. Once stimulation parameters are established, hemodynamic responses to ES will be monitored over several hours in those at risk of DVT. Intermittent ES could be initiated upon admission to an acute care facility and accompany an individual from the hospital to their home or rehabilitation setting. In addition, intermittent ES has other potential benefits such as preservation of range of motion, muscle strength and reduction of spasticity. Such benefits may increase its utility by possibly preventing secondary complications related to bed rest or paralysis.

Disclosures: **E.N. Ravid:** None. **S. Gray:** None. **V.K. Mushahwar:** None.

Poster

716. Stroke Recovery

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Topic: C.21.Stroke Recovery

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Title: Traditional Chinese medicine for intracerebral hemorrhage: A systematic review

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Abstract: Background and Purpose: Traditional Chinese medicine (TCM) are widely used for intracerebral hemorrhage (ICH) in China. The aim of this study was to systematically evaluate clinical effects of TCM for ICH. **Methods**: We identified all TCM that were listed in the Chinese National Essential Drug list of 2012 and those commonly used TCM in current clinical practice for ICH. Twenty-six TCM were identified for further evaluation. An extensive search including PubMed, EMBASE, CBM,CNKI and the Cochrane Library was performed up to November 2013. We searched for reports of randomized controlled trials on any of the 26 TCM for ICH comparing one TCM with control. Primary outcomes included death and adverse events. Effects on neurological impairments were a secondary outcome. **Results**: Three-hundred sixty trials (34398 patients) on 22 TCM were available and included. All these trials were conducted in China and only one trial was published in journal of the Neurological Sciences,the others were published in Chinese journals. The methodological quality of included trials was generally "poor." Few trials reported methods of randomization. The results indicated that TCM significantly decreased the number of death compared to the control group (OR 0.41, 95% CI 0.35 to 0.49, P<0.00001) in seventy-five trials on 14 TCM in primary outcomes. The adverse

events were found no statistically significant difference between 2 groups in seventeen trials on 8 TCM. For secondary outcome, two-hundred eighty-eight trials measured neurological deficit at the end of treatment. TCM significantly improved neurological deficit (OR 3.11, 95% CI 2.91 to 3.32, P<0.00001) on all TCM. Of the 22 TCM, 9 drugs (San Qi, Xing Nao Jing, Dan Shen, Qing Kai Ling, Yin Xing Ye, Ci Wu Jia, Deng Zhan, Nao Xue Kang and Liang Xue Tong Yu) had relatively more studies and patient numbers. **Conclusions**: The evidence currently available showed that TCM may decrease the risk of death and can also reduce neurological deficit in patients with ICH.However, more high-quality trials are needed. Nine drugs could be further research priorities.

Disclosures: Y. Zhang: None. **Q. Zhang:** None. **Y. Sun:** None. **J. Li:** None. **L. Wei:** None.

Poster

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Topic: C.21.Stroke Recovery

Support: NINDS (NS074559)

Title: Prophylactic edaravone treatment against transient cerebral hypoxia-ischemia-implications for postoperative stroke and encephalopathy

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Abstract: Stroke is one of the most dreaded complications of surgery, including the coronary artery bypass grafting (CABG). Besides cardiogenic emboli, the combination of transient cerebral hypoxia and ischemia during surgery may induce thrombosis leading to stroke and cognitive dysfunction. However, due to the risk of hemorrhagic complications associated with the use of anticoagulants, there has been no well-accepted prophylactic medication against this condition. In this study, we test whether prophylactic administration of edaravone, a potent free radical scavenger that has been approved in Japan for treating acute ischemic stroke, confers protection against experimental transient hypoxia-ischemia (tHI)-induced cerebral infarction. In the tHI-induced thrombotic stroke model (Sun et. al., PLOS ONE, 2014), we challenge adult C57Bl/6J male mice with 30-min ligation of the right common carotid artery plus systemic

hypoxia (7.5% O2). Interestingly, while 30-min unilateral carotid occlusion or systemic hypoxia by itself causes no discernible brain damage, the combination of both insults triggers endogenous thrombosis and causes mortality or hemispheric infarct. Yet, prophylactic administration of edaravone (4.5 mg/kg x 2, 1 h before and 1 h after tHI, IP) significantly decreased the mortality rate (from 38% in vehicle-controls to 13% in edaravone-treatment) and infarct size ($p<0.001$). The prophylactic edaravone therapy also improved post-tHI brain oxygen saturation (SaO2 84% in vehicle-controls versus 103% in edaravone-treatment at 1 h recovery) and prevented cerebral blood flow reperfusion deficits (65% in vehicle-controls versus 85% in edaravone-treatment at 2 h recovery). Prophylactic edaravone also reduced tHI-induced de-phosphorylation of eNOS at the Ser1177 residue, an indicator of its activity, and decreased the deposition of fibrin, platelets, and exposure of P-Selectin along the cerebral blood vessel wall. Importantly, these protective effects were disappeared if the same dose of edaravone was administered at 3 and 4 h post-tHI, suggesting a critical therapeutic window flanking or immediately after the tHI insult. Together, these results suggest that prophylactic administration of edaravone confers robust protection against transient cerebral hypoxia-ischemia in experimental models. Given the clinical experience of good safety and no toxicity for edaravone in acute ischemic stroke, we suggest that prophylactic edaravone may be considered as a prophylactic medication prior to major surgery, especially for individuals with chronic carotid stenosis.

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Poster

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Topic: C.21.Stroke Recovery

Support: NIDRR Grant H133G070089

Title: Wide pulse neuromuscular electrical stimulation increases hand opening in individuals with moderate to severe stroke

Authors: ***J. YAO**[1], F. MAAGDENBERG[2], Y. LAN[1], J. E. SULLIVAN[1], J. DEWLAD[1]; [1]Physical Therapy & Human Movement Sci., Northwestern Univ., Chicago, IL; [2]Dept. of Biomechanical Engin., Delft Univ. of Technol., Delft, Netherlands

Abstract: Objective: Many individuals with moderate to severe stroke cannot open their paretic hand sufficiently for function. Wide Pulse Neuromuscular electrical stimulation (WP-NMES) to targeted muscles has been reported to increase the desired forces in individuals with stroke. Furthermore, it has been shown that a Wide Pulse Low Frequency NMES (WPLF-NMES) following a Wide Pulse High Frequency NMES (WPHF-NMES) creates higher forces than the WPLF-NMES preceding the WPHF-NMES. However, the actual hand-opening distances achieved by WPHF-NMES and by the subsequent WPLF-NMES are still unknown. This study investigated effects of WPHF-NMES and of the subsequent WPLF-NMES on hand opening distance in our target population.Methods: We recruited 13 individuals with moderate to severe arm paresis following stroke. They performed maximal hand opening with assistance from NMES delivered with three different stimulation patterns: 1) 25 Hz frequency, 250 μs pulse duration for 9s; 2) variable rate 25-100-25 Hz, 500μs pulse duration; and 3) with 1000 μs pulse duration, 3 s/phase. The amplitude of stimulation for all conditions was set as 1.5 times of the motor threshold. Two co-registered Optotrak cameras measured hand-opening distance between the tip of thumb and index finger. For each stimulation pattern, the maximal hand-opening distance during each of the stimulation phases was quantified and normalized by the maximal physically allowed opening distance. Results: A one-way (3 NMES patterns) repeated measure of ANOVA demonstrated that NMES parameters have a significant effect on hand-opening in individuals with moderate to severe stroke ($F=13.4$, $p<0.001$). The hand opening achieved by the stimulation pattern 3 was larger than the other two patterns, and was about 72mm. Using this pattern, a one-way (3 phases) repeated measure ANOVA demonstrated a significant effect of phase. A paired t-test showed that both 100 Hz and second phase of 25 Hz stimulation created significantly larger hand opening as compared to that achieved by the first 25 Hz stimulation ($p<0.005$). No significant difference in opening distance between the 100 Hz and second 25 Hz was found ($p=0.7$). Our results suggest that alternative pattern between WPHF-NMES and WPLF-NMES may be useful in achieving a greater hand-opening in individuals with moderate to severe hand paresis post stroke, while reducing the possibility of muscle fatigue.

Disclosures: J. Yao: None. **F. Maagdenberg:** None. **Y. Lan:** None. **J.E. Sullivan:** None. **J. Dewlad:** None.

Poster

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Support: Flory Foundation

Title: Low-level acoustic speech features correlate with language measures in aphasia therapy

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Abstract: Assessing change during language therapy for chronic aphasia (acquired language disorder resulting from brain damage) is difficult because improvement is incremental and existing measures are time consuming. Moreover, language therapy may modify verbal productions on multiple levels, for example a treatment targeting grammatical structures may decrease phonological errors or speech fluency. Whereas detailed analysis of speech and language production cannot be done in real time, automated (real time) measurements of speech production that may correlate with fluency characteristics of speech production in aphasia can be useful. For two participants (P1, P2) with chronic non-fluent aphasia we combined automated acoustic analysis of treatment sessions with a manual measurement of *speech efficiency,* namely, lexical content over time ("good" words/minute [wpm]). With Sound Analysis Pro (SAP2011) we obtained simple temporal and spectral features of the language samples: *% vocalizations* (*%vocal*) = proportion of vocal segments vs. silence; and *spectral complexity* (*spectral*) = variance of wiener entropy (noisiness) within a vocal segment, averaged across all segments. For both P1 & P2 speech efficiency was correlated with *%vocal* and *spectral* (P1: r= .62 and r = .52 respectively, P2: r= .86 and r = .54 respectively) and the combined acoustic score (product of *%vocal* and *spectral*) was highly correlated with speech efficiency: P1 r = .63 and P2 r = .75. Acoustic scores correlated better with "good" wpm, and more weakly with a simple count of words/minute (which does account for lexical accuracy): *% vocal* (r = .75 vs. .68 for good wpm vs. wpm respectively); *spectral* (r = .53 vs. .41). Following 20 hours of treatment, P1 produced significantly more accurate verbs and grammatical sentences following treatment. Similarly, automated acoustic analysis showed P1's increased in mean duration and spectral complexity from session 1 to session 20: mean duration: 503$\pm$46 to 694 $\pm$ 39, p <.05; spectral complexity: 1.17$\pm$0.08 to 1.47$\pm$ 0.08, p <.05. Further, the combined acoustic measure was highly correlated with treatment session number (r = .71). P2 did not improve on language or acoustic measures following treatment. In sum, automatically obtained low-level acoustic features of speech correlated with a number of language measures of the same productions in two people with aphasia. If this effect is replicated in a larger sample, automated acoustic measures recorded continuously during the course of aphasia treatment would allow dynamic adjustment of treatment strategy and performance feedback potentially enhancing treatment outcomes.

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Poster

716. Stroke Recovery

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Topic: C.21.Stroke Recovery

Support: NINDS P01 NS 19632

Title: Does earlier age of onset predict better outcome from stroke?

Authors: *K.-H. CHEN, K. MANZEL, Y. TORRES, N. AKSAN, N. DENBURG, D. TRANEL, S. W. ANDERSON;
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Abstract: There is considerable evidence that overall neural plasticity diminishes with age. Younger animals tend to have better recovery of motor function following cerebral injury than do older animals, a finding referred to as the "Kennard Principle". In humans, the Kennard Principle is supported by the finding of better recovery from aphasia acquired in childhood as compared to adulthood, but there is evidence that this principle may not apply to neural systems underlying emotion and social behavior. To date, there has been little study of age-of-onset effects across cognitive domains, or that has taken into account other factors that may contribute to the degree of functional recovery after brain injury, such as sex and years of education. The present study examined the relationship between age of stroke onset and cognitive outcome after controlling for demographic factors (sex, years of education), age at the time of testing and stroke type (ischemic or hemorrhagic). Participants included 464 patients (249 men and 215 women) who suffered a single stroke (295 ischemic and 169 hemorrhagic). Age of stroke onset ranged from 0 to 82 years old. Years of education ranged from 7 to 20 years. Cognitive outcomes include: 1) Language, assessed by Boston Naming Test and Controlled Oral Word Association. 2) Executive function, assessed by Trail Making Test Part B and Wisconsin Card Sort Test. 3) Memory, assessed by Rey Auditory Verbal Learning Test and Benton Visual Retention Test. 4) Visuospatial function, assessed by Complex Figure Test-Copy and Judgment of Line Orientation Test. Raw and normalized test scores were analyzed separately using linear regression models. Analysis of the raw scores without controlling for any other factors suggested a relationship between age of stroke onset and cognitive outcomes in most domains. However, when demographic factors, age of testing and stroke type were controlled, age of stroke onset was no longer predictive of any outcome in any domain. Similarly, when the standardized test scores

were normalized for age and education, age of stroke onset was not predictive of any cognitive outcome. These findings suggest that, across multiple domains of cognition, age of stroke onset is not a strong predictor of functional outcome.

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Poster

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VA RR&D CofE

Title: Combining treadmill training with overground training and rhythmic auditory cueing for individuals with chronic stroke: Is there a benefit?

Authors: ***J. WHITALL**, S. MCCOMBE WALLER, A. HOWE, C. HAFER-MACKO, L. FORRESTER;
Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Individuals with chronic stroke are interested in maintaining their ability to move around the community. Treadmill training of sufficient length is one intervention shown to increase comfortable walking speed as well as endurance for walking immediately after training. However, it does not increase symmetry, which is a goal of many stroke survivors. Furthermore, retention of any changes from these long-term interventions has not been reported. Here we ask whether 6 weeks of treadmill training followed by 6 weeks of circuit overground training is sufficient to induce changes in walking speed and endurance that are maintained after 12 weeks of no training. We also asked whether randomly assigning the addition of rhythmic auditory cueing (RAC), while training, to half the participants would result in improved quality of walking such as increased step-time symmetry and reduced stride-time variability. Nine participants with chronic stroke (mean age = 50.7; mean walking speed = 0.54 m/s) completed the protocol. None were involved in previous studies of treadmill training. Using the Wilcoxon

Sign rank test we found an immediate mean increase in comfortable walking speed of 0.18m/s, and in fast walking speed of 0.14m/s as measured on an instrumented gaitmat. Also the mean maximum distance covered in a six-minute walk test increased by 186 feet. These increases were all maintained 3 months later. Inspection of those individuals who received cueing vs. those who did not revealed a tendency for improvement after cueing of step-time symmetry ratio of 0.213 vs. -0.031 and reduction of stride-time coefficient of variability for paretic leg of 0.031 vs. 0.003 and for non-paretic leg of 0.018 vs. 0.001. Although the group effects for the RAC group were only trends, owing to one person, these trends were also durable. Taken together these preliminary findings suggest that some chronic stroke survivors can benefit from the addition of rhythmic cueing to standard exercise protocols. The increased symmetry and decreased variability are associated with a reduction of fall risk. In addition to a larger trial, understanding who can benefit from auditory cueing and why they benefit are future research directions.

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Poster

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Support: NIH Grant 1K01HD069504

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Title: Multimodal predictors of rehabilitation related recovery in stroke

Authors: *N. VARNERIN[1], D. A. CUNNINGHAM[1,4], S. ROELLE[1], K. A. POTTER-BAKER[1], V. SANKARASUBRAMANIAN[1], K. SAKAIE[2], E. BEALL[2], A. MACHADO[3], E. B. PLOW[1]; [1]Dept. of Biomed. Engin., [2]Imaging Ctr., [3]Ctr. for Neurolog. Restoration, Cleveland Clin., Cleveland, OH; [4]Kent State Univ., Kent, OH

Abstract: Stroke is the leading cause of upper limb disability. Unfortunately, variability in stroke location and impairment results in different rates of recovery. Therefore, in order to accommodate the wide patient variability, therapists often use functional tests to aide in development of individualized therapy. However, while baseline function is significant in

predicting recovery, it is often limited since patients with the same level of impairment can improve via different neural processes. Knowing the unique process of recovery would allow one to individualize therapies for better outcomes. Therefore, here we investigated if initial assessments of corticospinal integrity (diffusion tensor imaging), corticospinal output (transcranial magnetic stimulation), interhemispheric inhibition (ipsilateral silent period), or cortical activation (functional magnetic resonance imaging) could be predictors of recovery following a five week therapy session. Recovery was defined in terms of impairment, dexterity, and perceived disability. Stepwise linear regressions were used to find predictors of recovery. In addition, we also determined if patients' neural substrates could be predictors of long-term (3 month) follow-up recovery. Notably, we found that predictors of alleviating impairments were both corticospinal integrity (β=0.537, R2=0.975, p=0.001) and corticospinal output (β = -0.604, R2=0.975, p=0.001). Corticospinal integrity and corticospinal output were also found to be predictors of perceived disability, but only after long-term follow-up (β=0.814, R2=0.999, p=0.001; β=0.613, R2=0.999, p=0.003). Finally, we identified interhemispheric inhibition as the only significant predictor of dexterity improvement (β=0.781, R2=0.61, p=0.013). It is important to note that cortical activation did not show significant contribution to recovery. . However, while baseline function is significant in predicting recovery, the only baseline test found to be a predictor of recovery was perceived disability (β=0.726, R2=0.527 p=0.027). Therefore, the results from our study suggest that corticospinal output and integrity may be a better measure to predict reduction of impairments in stroke patients. Likewise, recovery of dexterity may be best predicted by interhemispheric inhibition. Therefore, future work should consider including measures of corticospinal integrity, corticospinal output, and interhemispheric inhibition to further tailor therapy to individual patients' neural processes.

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Poster

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Title: Paradoxical motor recovery from a first stroke by re-opening a sensitive period with a second stroke

Authors: *S. ZEILER[1], R. B. HUBBARD[2], E. M. GIBSON[2], K. NG[3], T. ZHENG[1], R. J. O'BRIEN[2], J. W. KRAKAUER[2];
[2]Neurol., [1]Johns Hopkins, Baltimore, MD; [3]Neurol., UCLA, Los Angelas, CA

Abstract: After stroke, there is a time-limited period of increased responsiveness to training due to heightened plasticity, which is thought to be induced by ischemia itself. Using a mouse model, we have previously shown that most training-associated recovery after a caudal forelimb area (CFA - rodent primary motor cortex) stroke occurs in the first week and is attributable to reorganization in the medial premotor area (also called agranular medial cortex - AGm). The idea of a stroke-induced sensitive period leads to the counterintuitive prediction that a second stroke should reopen this window and lead to paradoxically enhanced recovery from the first stroke. To test this prediction, we induced a focal stroke in the medial premotor area of mice with incomplete and plateaued recovery after a focal CFA stroke. This second stroke led to a dramatic response to early post-stroke training with recovery to normal performance. Together, these data indicate that ischemia can re-open a sensitive period and mediate recovery from an earlier stroke. Future work will need to characterize what the critical molecular pathways are that ischemia triggers.

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Poster

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Bromedicon

Title: Acute reemergence of neurological function following 4R treatment after ischemic stroke

Authors: *W. CASTRO[1], H. MARTINS[2], P. FERCHMIN[2], G. GRUDZIAK[3], V. ETEROVIC[2]; [1]Univ. Central Del Caribe, Cayey, Puerto Rico; [2]Univ. Central Del Caribe, Bayamon, Puerto Rico; [3]Bromedicon, Yardley, PA

Abstract: Background: Stroke is one of the leading causes of disability in adults. Neuroprotective compounds that increase the opportunity of recovery have yet to be described. 4R-cembranoid (4R), a compound isolated from tobacco, displays neuroprotective activity against different types of insults to the brain, including the neurodegeneration induced by organophosphate and by ischemic events. This work studied the effect of 4R on the neurological damage induced by ischemic stroke in rats, using electrophysiological, cerebral blood flow (CBF) and histological methods. **Methods**: Male Sprague Dawley rats were utilized as a stroke model using transient middle cerebral artery occlusion (MCAO). Two hours after MCAO, the rats received a single injection of 4R (6 mg/kg, sc) or vehicle (DMSO). Somatosensory evoked potentials (SSEP) were measured at various times and electroencephalograms (EEG) were measured continuously before and after MCAO. Histology assays were performed on 15 μm thick brain slices to assess neuronal damage and repair. **Results:** The amplitude of SSEP and frequency of EEG recorded from the ipsilateral hemisphere was significantly decreased after MCAO by comparison with pre-operation values, both in 4R and control groups. 4R treated animals exhibit a acute reemergence of SSEP amplitude and increase in EEG frequency during the first 48 hours while vehicle treated animals did not exhibit any SSEP amplitude from the infarcted hemisphere during the same time. In the contralateral hemisphere, both groups displayed an increase in amplitude but that increase was larger in the 4R group. Subjects included in these results were those who exhibit over 70% decrease of CBF during the MCAO for both 4R and vehicle treated animals. Sham operated animals did not exhibit changes in CBF, SSEP amplitude or EEG frequency. **Conclusion:** 4R is a promising neuroprotective compound that promotes the protection not only of brain tissue but of function as well.

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Poster

716. Stroke Recovery

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Topic: C.21.Stroke Recovery

Title: Bursting and regular chronic cerebellar stimulation modulates perilesional cortical angiogenesis and neurogenesis

Authors: *H. H. CHAN[1,2], J. COOPERRIDER[2], J. GALE[2], A. MACHADO[2];
[2]Neurosci., [1]Cleveland Clin., Cleveland, OH

Abstract: Stroke is the leading cause for disability in the industrialized world. New treatments to improve post-stroke motor recovery are needed. Previous finding from this group suggests that chronic electric stimulation to lateral cerebellar nucleus (LCN) of rats induces synaptogenesis that may be one of the underlying mechanisms of recovering the motor function. In this study, we proved that there is also neurogenesis and angiogenesis after the chronic electrical stimulation and intermittent burst to the LCN. A total of 24 adult male Long Evans rats underwent unilateral endothelin-1 lesion in the motor area of dominant cerebral cortex and placement of a stimulating electrode in the contralateral LCN. Experimental rats were separated into 4 groups, namely 2-week non-stimulation (2NS), 2-week stimulation (2S), 6-week non-stimulation (6NS) and 6-week stimulation (6S). At 2 or 6 weeks post-ischemia, the treatment phase was initiated with rats in stimulation groups receiving pulsed 30-Hz stimulation plus 100Hz intermittent burst for 12 hours/day and with also administration of 50mg/kg 5'-bromo 2'-deoxyuridine (BrdU) for labeling dividing cells, whereas non-stimulation groups received only BrdU. 4 week after treatment, immunohistochemistry were then performed to reveal the stimulation mediated cell growth in the perilesional area. There is a significant increase in the perilesional number of angioblasts (CD105) and neural progenitor cells (doublecortin) and total neurones (NeuN) labeled also by BrdU in stimulation groups (both 2S and 6S) when compared to non-stimulation groups (2NS and 6NS), indicating that LCN stimulation facilitates the angiogenesis and neurogenesis. Morphologically, LCN stimulation promotes the differentiation of angioblasts to mature endothelial cells for forming new vessels. Furthermore, LCN stimulation induces an increase of astrocytes (GFAP) in the perlesional area, suggesting that the astrogliosis might associate with the angiogenesis and neurogenesis. These results indicate that delayed and chronic electrical activation of ascending cerebellofugal pathways enhances the angiogenesis and neurogenesis after focal cortical ischemia in rat. The recovery was associated with an increase in perilesional cortical plasticity relative to nontreated controls. Possibly, these could restore the compromised neural function at motor area of cortex and hence the motor functions.

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Poster

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Title: Differential modulation of the lateral cerebellar nucleus during successful and unsuccessful reaching attempts

Authors: *J. COOPERRIDER, J. T. GALE, R. GOPALAKRISHNAN, C. WATHEN, H. CHAN, H.-J. PARK, A. MACHADO;
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Abstract: Our group has previously demonstrated that beta band stimulation of the lateral cerebellar nucleus (LCN) has a neurorehabilitative effect on skilled reaching performance of the ischemic rodent. This project intends to investigate the electrophysiology of the rat LCN during the performance of a skilled reaching task in order to better inform therapeutic stimulation paradigms. We hypothesize that stimulation delivered to the LCN in a "pseudo-natural" way may further increase recovery after stroke. A five-electrode array was advanced into the LCN of seven rats. Following a one-week recovery period, animals were trained to reach for sugar pellets with the paw ipsilateral to the implanted array. Local field activity was recorded wirelessly from each electrode while the rodent performed the reaching task. Behavioral training was concurrently videotaped, allowing for the classification of reaching attempts into hits and misses. Analysis revealed that there was a significant increase in beta band activity during the reaching attempt, comparing missed trials to hits. In addition, an increase in high-gamma band activity corresponding to the grasp of the pellet was present in hits trials but absent in misses. These results demonstrate that there are significant oscillatory differences in LCN activity during successful and unsuccessful reaching attempts and implicate beta band activity as a potential error correction signal.

Disclosures: J. Cooperrider: None. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cardionomics, Enspire, Functional Neuromodulation, Ati, Boston Scientific. **J.T. Gale:** None. **R. Gopalakrishnan:** None. **C. Wathen:** None. **H. Park:** None. **H. Chan:** None.

Poster

716. Stroke Recovery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 716.19/CC36

Topic: C.21.Stroke Recovery

Support: NIH Grant 1R43NS084566-01

Title: Vagus nerve stimulation and healthy limb training modify stroke recovery

Authors: *A. NGUYEN[1], N. KHODAPARAST[2], S. HAYS[3], M. P. KILGARD*[4], R. L. RENNAKER II*[5];
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Abstract: Stroke can cause impairments in the upper extremities. Vagus nerve stimulation (VNS) is a safe, tolerable treatment that has been shown to drive powerful, long-lasting plasticity especially when paired with somatosensory inputs. VNS paired with rehabilitative training has been shown to improve functional recovery beyond what can be achieved by rehabilitative training alone in models of stroke. Though preclinical studies of stroke have repeatedly shown the beneficial effect of VNS on stroke recovery, clinical trials involving pairing VNS with movements (or targeted plasticity therapy) after stroke in humans have not been as successful. Early post-stroke rehabilitation by way of occupational therapy often includes extensive training of the unimpaired forelimb which can provide some immediate functional benefits, however recent studies suggest that this training may interfere with functional recovery of the paretic forelimb. To test this hypothesis, rats will be trained on a novel force generation task, the Isometric Pull task, to asymptotic performance. Upon attainment of task proficiency, rats will receive a cortical/subcortical ischemic lesion in motor regions, after which the rats will be trained using their nonparetic forelimb. Following nonparetic forelimb training motor recovery of the paretic forelimb will be assessed. VNS will be paired with rehabilitative training. Control animals will perform rehabilitative training without VNS. After the completion of therapy, intracortical microstimulation will be employed to develop high-resolution maps of the cortical representations of peripheral movements. The effects of training the nonparetic forelimb in will further the understanding of the neural mechanisms of VNS promoted stroke recovery.

Disclosures: **A. Nguyen:** None. **N. Khodaparast:** A. Employment/Salary (full or part-time):; Microtransponder Inc.. **S. Hays:** None. **M.P. Kilgard*:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Microtransponder Inc. **R.L. Rennaker II*:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Vulintus Inc..

Poster

716. Stroke Recovery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 716.20/DD1

Topic: C.21.Stroke Recovery

Support: NIH Grant 1R43NS084566-01

Title: Translational potential of vagus nerve stimulation to enhance recovery of motor function after stroke

Authors: *N. KHODAPARAST[1], R. CASAVANT[2], S. A. HAYS[3], A. RUIZ[3], N. JONES[3], B. NGUYEN[3], M. THOMAS[3], C. LE[3], R. RENNAKER, II[3], M. P. KILGARD[3];
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Abstract: Stroke is the second most common cause of disability worldwide. Following a stroke only 30% of patients regain some degree of use of their upper extremity, even after intensive rehabilitation therapy. Neuroplasticity within motor circuitry is believed to support recovery of function after stroke. We have recently developed a method using VNS paired with motor training to drive robust, specific plasticity in the motor cortex. Based on this enhancement of plasticity, we speculated that VNS could facilitate post-stroke motor recovery. Our recent studies indicated that VNS paired with rehabilitative training significantly enhances recovery of multiple measures of forelimb strength and movement speed after cortical ischemic stroke. To further the translation potential of our therapy, we tested the effects of VNS paired rehabilitative training in a chronic model of cortical/subcortical ischemic stroke. Following the ischemic lesion, rats returned to their home cage for 3 weeks, and did not begin rehabilitative training until 5 weeks post-lesion. Our results indicate that VNS paired with rehabilitative training may enhance recovery of forelimb function in chronically impaired rats.

Disclosures: **N. Khodaparast:** A. Employment/Salary (full or part-time):; Microtransponder Inc. **R. Casavant:** A. Employment/Salary (full or part-time):; Microtransponder Inc.. **S.A. Hays:** None. **A. Ruiz:** None. **N. Jones:** None. **B. Nguyen:** None. **M. Thomas:** None. **C. Le:** None. **R. Rennaker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vulintus Inc. **M.P. Kilgard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Microtransponder Inc.

Poster

716. Stroke Recovery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 716.21/DD2

Topic: C.21.Stroke Recovery

Support: Michael J Fox Foundation RRIA

NIH NIDCD R01 DC010433

Title: Vagus nerve stimulation paired with rehabilitative training improves recovery of forelimb function in clinically relevant models of stroke

Authors: *S. A. HAYS[1], N. KHODAPARAST[2], A. RUIZ[2], M. IYENGAR[2], P. DAS[2], E. NUTTING[2], I. KUSHNER[2], V. LAND[2], N. HOUSHMANDI[2], R. RENNAKER, II[2], M. KILGARD[2];
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Abstract: Stroke is a debilitating neurological disease that affects 800,000 individuals in the United States each year, with many of these patients suffering chronic motor impairments. Neuroplasticity within motor circuitry is believed to support recovery of function after stroke. We have recently developed a method using vagus nerve stimulation (VNS) paired with motor training to engender phasic release of neuromodulators and drive robust, specific plasticity in the motor cortex. Our recent studies indicate that VNS paired with rehabilitative training significantly enhances recovery of multiple measures of forelimb strength and movement speed after cortical ischemic stroke. We sought to further evaluate the efficacy of VNS in two severe models of stroke that are reflective of the clinical population of stroke patients. Here we report the use of VNS in a model of subcortical intracerebral hemorrhage that includes white matter damage and in a model of ischemic stroke in aged rats. One cohort of rats was trained to perform the bradykinesia assessment task and then received an intracerebral hemorrhage. At least nine days after lesion, rats began rehabilitative training with or without VNS. VNS paired with rehabilitative training resulted in significantly greater recovery (77% recovery of initial impairment) of forelimb movement speed than rehabilitative training without VNS (29% recovery). Our preliminary data indicates that forelimb-specific reorganization within the ipsilesional motor cortex correlates with recovery. A second cohort of rats, aged 16 months, was trained to perform the isometric force task and then received a cortical ischemic lesion. After at least nine days, rats underwent rehabilitative training with or without VNS. VNS paired with

rehabilitative training conferred significantly enhanced recovery of forelimb strength (98% recovery) compared to rehabilitative training without VNS (47% recovery). We are now investigating the role of structural plasticity in motor cortex and in corticospinal projections to support VNS-dependent recovery. The results from these studies provide additional support for VNS as a potential post-stroke intervention to improve recovery of motor function.

Disclosures: **S.A. Hays:** None. **N. Khodaparast:** A. Employment/Salary (full or part-time):; MicroTransponder, Inc.. **A. Ruiz:** None. **M. Iyengar:** None. **P. Das:** None. **E. Nutting:** None. **I. Kushner:** None. **V. Land:** None. **N. Houshmandi:** None. **R. Rennaker:** A. Employment/Salary (full or part-time):; Vulintus, Inc. **M. Kilgard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MicroTransponder, Inc..

Poster

716. Stroke Recovery

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 716.22/DD3

Topic: C.21.Stroke Recovery

Support: PROAPARC/UNASP-SP

Title: Shiatsu and recovery of functional capacity in post-stroke patients

Authors: ***J. H. SATO**[1,2], S. M. TAGAMI[2], R. H. D. D. LABRONICI[2], F. M. ALFIERI[2], R. N. ISAYAMA[2];
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Abstract: Stroke is an important cerebrovascular disease to cause morbidity and mortality in Brazil and worldwide. Motor function and cognition are mostly impaired in post-stroke patients, resulting in reduced functional capacity that compromises their quality of life. Functional and emotional recovery is variable and depends on the severity of the injury, socio-cultural factors and therapeutic strategies. Shiatsu is an ancient technique of complimentary therapy based on non-invasive points of pressure that are usually at the anatomical sites of muscle-tendon transition or close to motor points where muscles are effectively stimulated by electrodes. Finger pressure using as a shiatsu technique employed on spastic muscles could ameliorate spasticity by stimulating Golgi tendon organs. Such structures, specially regulate muscular tone of rigid

muscles. Preview studies suggest that shiatsu mitigates sequel of stroke. Although cerebrovascular injuries cause primary lesion of the upper motor neuron, interventions in peripheral neuromuscular structures may also benefit muscle tone and performance, by increasing the functionality and quality of life of these patients. The aim of this study was evaluating the beneficial effects of shiatsu on functional capacity and quality of life in post-stroke chronic patients. This study was approved by the ethics committee. Six (n=6) post-stroke patients were assigned for shiatsu-kinesiotherapy group (SHI) with pressure points in upper and lower limbs. A control-kinesiotherapy group (CRT, n=6) received conventional kinesiotherapy and both groups were treated twice a week for four months. Patients responded to SF-36 questionnaire and Barthel index prior and after all therapeutic procedures. The results showed that shiatsu and/or kinesiotherapy did not change blood pressure, mood, consciousness or caused discomfort in post-stroke patients. SF-36 revealed that SHI group had improved their pain management, vitality, social and the mental health state ($p < 0.05$) comparing to its control. Barthel index showed a recovery in SHI group from severe to moderate dependence (index ≥ 60 and < 80), which did not occur in CRT. This study revealed a significant improvement for management of pain, vitality, social functioning and mental health domains in SHI as compared to its control. Barthel index demonstrated that SHI is functionally more independent than the CRT. It may be concluded that shiatsu associated with conventional kinesiotherapy in post-stroke patients promotes recovery of functional capacity as well as their quality of life.

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Poster

716. Stroke Recovery

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Program#/Poster#: 716.23/DD4

Topic: C.21.Stroke Recovery

Support: Richard Merkin Foundation for Neural Regeneration at UCLA

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: CREB facilitates recovery and reorganization of forelimb sensory map after a photothrombotic stroke in mice

Authors: *L. CARACCIOLO[1], M. MAROSI[1], Y. SANO[2], A. SILVA[2], C. PORTERA-CAILLIAU[1], T. CARMICHAEL[1];
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Abstract: In the brain, the transcriptional factor CREB (cAMP responsive element binding protein) play a key role in many intracellular signaling events that critically regulate neural functions such as neuronal plasticity, long-term memory formation and axonal sprouting in neurodevelopment.In this study, we tested the function of the CREB in neural repair after photothrombotic stroke in the mouse forelimb sensory cortex with a gain of function. We transfected a small number of neurons in motor cortex of wild type (WT) mice with a lentivirus that overexpresses CREB in excitatory neurons (pCamk2a_F2A_EGFP/CREB). Previously we reported that lentiviral CREB transfection improves motor recovery after stroke. The goal of the studies is to determine if this promotes remapping of cortical sensory representations.Intrinsic Optical Signal mapping (IOS) was performed over 8 week after a photothrombotic stroke. IOS mapping of WT mice transfected with pCamk2a_F2A_EGFP/CREB show an early reorganization of the forelimb sensory map starting at two weeks after stroke when compared to mice transfected with a control lentivirus (pCamk2a_F2A_EGFP/Tomato) where the reorganization of sensory forelimb map is observed at 8 weeks after stroke. These results indicate that increased CREB levels in the motor cortex facilitate the reorganization of sensory forelimb map after stroke. Our future studies will determine the change the gene expression changes induced by CREB-activated neurons during this period of enhanced sensory remapping. Understanding the role of CREB after stroke could determine the role of specific mechanisms in neural repair and recovery in motor control after stroke and consequently develop new treatments for this devastating disease.Supported by The Richard Merkin Foundation for Neural Regeneration at UCLA and Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

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Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.01/DD5

Topic: C.21.Stroke Recovery

Title: The tibial somatosensory evoked potential can prognosticate for the ambulation in hemiplegic stroke

Authors: *S. JEE;
Rehabil. Med., Chungnam Natl. Univ. Hosp., Daejeon, Korea, Republic of

Abstract: Introduction : To examine the association between tibial nerve somatosensory evoked potentials(SEP) and ambulatory outcomes in hemiplegic stroke patients. Methods : We reviewed medical records for hemiplegic patients with the first ever stroke who received inpatient rehabilitation from January 2009 to May 2013. We excluded the patient with diabetes, quadriplegia, bilateral lesion, brainstem lesion, age over 80 years, and severe musculoskeletal problem. Tibial nerve SEP were performed when they were transferred into our department. SEPs findings divided into three groups; normal, abnormal and absent response. According to the tibial SEP findings, Berg balance scale(BBS) and functional ambulation category(FAC) at discharge were compared among groups by the one-way ANOVA Result : Thirty one hemiplegic patients were included. BBS and FAC were significantly different according to the SEP findings(one way ANOVA, P<.001). Post-hoc analysis showed significant different between normal and absent response in BBS(P<.001) and FAC(P<.001), and between abnormal and absent response in BBS(P=.012) and FAC(P=.019). Functional outcomes of normal response group were better than abnormal group, but there was no statistical significance. Discussion : These findings suggest that initial tibial nerve SEP can be a useful biomarker for prognosticating functional outcomes in hemiplegic stroke patients.

Disclosures: S. Jee: None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.02/DD6

Topic: C.21.Stroke Recovery

Support: Toyota Motor Corporation Grant

Title: Phase synchrony of resting state electroencephalography in ischemic stroke: II. Distinct effects of lesion side on the relationship between hemispheric asymmetry in large-scale synchrony networks and functional recovery

Authors: *Y. UNO[1], T. KAWANO[2], N. HATTORI[1,2], M. HATAKENAKA[2], I. MIYAI[2], K. KITAJO[1];
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Abstract: Objectives: It is known that ischemic stroke breaks down large-scale EEG synchrony networks and brain functions. It remains unclear, however, whether there are any hemispheric effects of focal lesion on the relationship between synchrony networks and functional recovery. To address this issue, we investigated the effects of lesion side (i.e. left vs right) on EEG phase synchrony and functional recovery in ischemic stroke patients. Methods: Forty-four patients with focal ischemic stroke participated in the study after providing informed consent. We analyzed 19ch scalp EEG recordings during an eyes-closed resting condition using Phase Synchrony Index (PSI) to quantify the degree of EEG phase synchrony between distant cortical regions. An automated procedure was used to remove noisy epochs and patients. As a result, EEGs from forty-one patients (age = 66.7 ± 13.1 years, male/female = 30/11, left/right lesion = 18/23, days after onset = 40.7 ± 16.9) were used for further analyses.We computed Inter-, Left, and Right hemispheric phase synchrony statistics (IHPS, LHPS, and RHPS) as the mean of PSIs from all inter-, left (intra), and right (intra) hemispheric electrode pairs, respectively. We analyzed the correlations between the three statistics and the Functional Independence Measure (FIM), which is one of activities of daily living (ADL) measures, across individual patients with left or right lesions. We also analyzed MRI images to evaluate the lesion side and size. Results: We found significant correlations between FIM and IHPS, RHPS, or LHPS in the alpha band (8-13Hz) for the right lesion group ($p<0.05$). On the other hand, the left lesion group showed no significant correlations in the alpha band. In the low beta (14-17Hz), however, the right lesion group didn't show any significant correlations, whereas the left lesion group showed significant correlations between FIM and IHPS or LHPS ($p<0.05$). There was no significant difference in lesion size between the two groups. Conclusions: We found distinct effects of lesion side on the relationships between phase synchrony statistics and FIM in patients with postacute stroke. Focal lesions in the right hemisphere resulted in more globally impaired synchrony networks including intra-hemispheric synchrony networks in the intact (i.e. left) hemisphere at slower frequencies affecting functional recovery. The findings are consistent with the hypothesis that slower synchrony is suited for networking over long distances. The hemispheric asymmetry in impaired synchrony might account for disability in ADL caused by impaired lateralized functions such as motor, language, and attention.

Disclosures: **Y. Uno:** Other; Toyota Motor Corporation Grant. **T. Kawano:** Other; Toyota Motor Corporation Grant. **N. Hattori:** Other; Toyota Motor Corporation Grant. **M. Hatakenaka:** Other; Toyota Motor Corporation Grant. **I. Miyai:** Other; Toyota Motor Corporation Grant. **K. Kitajo:** Other; Toyota Motor Corporation Grant.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

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Topic: C.21.Stroke Recovery

Support: NIH/SCTR UL1 RR029882

NIH Grant C06 RR015455.

Title: Changes in diffusion mri following experimental stroke and rehabilitative training

Authors: *R. WEBER[1], X. NIE[2], E. S. HUI[5], J. H. JENSEN[2], M. F. FALANGOLA[3], J. A. HELPERN[3], D. L. ADKINS[4];
[1]Dept. of Neurosciences, [2]Ctr. for Biomed. Imaging; Dept of Radiology/Radiological Sci., [3]Dept of Neurosciences, Ctr. for Biomed. Imaging; Dept of Radiology/Radiological Sci., [4]Dept. of Neurosciences, Ctr. for Biomed. Imaging, Dept of Hlth. Sci. and Res., Med. Univ. of South Carolina, Charleston, SC; [5]Dept. of Diagnos. Radiology, The Univ. of Hong Kong, Pokfulam, Hong Kong

Abstract: Advanced diffusion MRI (dMRI), including diffusion tensor imaging (DTI) and diffusional kurtosis imaging (DKI), are highly sensitive to microstructural changes in brain. However, application of these techniques following stroke and rehabilitation has been limited. Previously, we found enhanced sensitivity of DKI metrics following acute stroke in the lesion core and peri-lesional area that correlated with glial and dendritic changes. Previous studies by our lab and others have demonstrated that motor training after brain damage induces functional motor recovery and neural plasticity in the peri-lesional remaining motor cortex. In the current study, we investigated the relationship between dMRI measures and rehabilitation-induced forelimb functional recovery. Adult male rats (3-4 months old) were trained to criteria of ~50% success on a skilled reaching task with their preferred limb and given an endothelin-1 (ET-1) induced unilateral ischemic stroke over the sensorimotor cortex (SMC) opposite the trained limb. Animals then received twenty-one days of impaired forelimb rehabilitative training (RT) on a reaching task or no rehabilitative training (No-RT). Animals were also assessed weekly on a battery of motor tasks. All animals underwent MRI scans prior to injury, 4 days post-injury, and after 21 days of RT or No-RT procedures, using a 7T Bruker Biospec MRI scanner. Diffusion-weighted images were acquired with 3 b-values (0, 1000, 2000 s/mm2) along 30 directions using TR/TE=4750/32.5ms, matrix=128x128, resolution=0.23x0.23x1mm3, and NEX=2. Diffusion and kurtosis tensors were calculated with Diffusional Kurtosis Estimator (DKE). Multi-slice

regions-of-interest (ROIs) were drawn in the lesion core and peri-lesional remaining motor cortex and in the forelimb region of the SMC in the non-infarcted hemisphere. Our data demonstrate that all animals have lesion-induced motor deficits following ET-1 strokes. RT improved forelimb function on the reaching task compared to No-RT. Peri-lesional DKI measures remained significantly different between the lesion and non-lesion SMC at four weeks post-injury, at which time DTI no longer detected hemispheric differences. Fractional anisotropy (FA) of the corpus callosum, inclusive of areas known to include SMC interhemispheric connections was higher following RT versus No-RT. These data indicate that DKI of peri-lesional motor cortex remains sensitive to chronic ischemic effects, unlike DTI-derived measures, and that skilled motor RT likely alters white matter connectivity between the two hemispheres.

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Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.04/DD8

Topic: C.21.Stroke Recovery

Support: Toyota Motor Corporation Grant

Title: Phase synchrony of resting state electroencephalography in ischemic stroke: I. Distinct effects of band frequency on various aspects of functional outcome

Authors: *T. KAWANO[1], N. HATTORI[1,2], Y. UNO[2], K. KITAJO[2], M. HATAKENAKA[1], H. YAGURA[1], H. FUJIMOTO[1,3], T. YOSIOKA[1], M. NAGASAKO[1], H. OTOMUNE[3], I. MIYAI[1]; [1]Neurorehabilitation Res. Inst., Morinomiya Hosp., Osaka, Japan; [2]Rhythm-based Information Processing Unit, RIKEN BSI-Toyota Collaboration Ctr., Riken, Saitama, Japan; [3]Dept. of Neurol., Osaka Univ. Grad. Sch. of Med., Osaka, Japan

Abstract: Objective: Focal brain lesions due to stroke can cause remote effects on neural networks. A recent study applied EEG Phase Synchrony (PS) analysis for the prediction of functional outcome after stroke (Wu et al, Clin.Neurophysiol.2011). While this pioneer study demonstrated a significant relationship between PS measure and functional outcome, only NIH Stroke Scale was used for the assessment, and clinical relevance of PS measure in stroke has not

been fully evaluated yet. The aim of the current study was to investigate clinical relevance of PS measure to detect network abnormalities after stroke by comparing it with various clinical scales for the assessment of impairment, ADL and cognitive functions. Methods: Nineteen patients with postacute ischemic stroke (mean age 67.5 ± 10.1 years, 5 women) admitted for inpatient rehabilitation were enrolled. After clinical routine EEG measurement, noisy epochs were eliminated by manual processing. Inter-Hemisheric Phase Synchrony (IHPS) of six frequency bands (delta, theta, alpha, low beta, beta, and gamma) was computed, and correlations of IHPS with clinical scales including the Functional Independence Measure (FIM) and its motor and cognitive subscales, and Mini Mental State Examination (MMSE) were investigated. In eight subjects with Unilateral Spatial Neglect (USN), Behavioral Inattention Test (BIT) was also evaluated. In ten subjects, EEG as well as FIM were measured on admission and at discharge for longitudinal analysis. Average interval of EEG measurements was 125.4±45.1 days. Spearman's correlation coefficient ρ was used to assess the relationship between PSI and clinical scales. Results: IHPS significantly correlated with motor FIM in the alpha band ($\rho = 0.818$, $p < 0.01$) and in the low beta band ($\rho = 0.560$, $p < 0.05$). IHPS correlated with cognitive FIM in the delta band ($\rho = -0.472$, $p < 0.05$), in the alpha band ($\rho = 0.664$, $p < 0.01$) and in the low beta band ($\rho = 683$, $p < 0.01$). IHPS correlated with FIM in the alpha band ($\rho = 0.756$, $p < 0.01$) and in the low beta band ($\rho = 0.607$, $p < 0.01$).Concerning cognitive functions, IHPS correlated with MMSE in the low beta band ($\rho = 0.643$, $p < 0.01$). IHPS from subjects with USN showed correlation with BIT in the low beta band ($\rho = 0.826$, $p < 0.05$). FIM and IHPS significantly increased at discharge ($p < 0.01$ two-tailed paired t test). Conclusion: We have found distinct effects of band frequency on various aspects of functional outcome after ischemic stroke. We also revealed that changes of IHPS paralleled improvement of ADL in the longitudinal assessment. These results suggest that modulation of neuronal network assessed by IHPS can be a surrogate marker of functional recovery after stroke.

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Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

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Program#/Poster#: 717.05/DD9

Topic: C.21.Stroke Recovery

Support: NSERC Grant 418489

CIHR Grant 126127

Title: Changes in gangliosides expression detected by MALDI IMS in a combined rat model of Alzheimer's disease and stroke

Authors: *S. CAUGHLIN[1], J. D. HEPBURN[1], D. PARK[1], K. JURCIC[2], K. YEUNG[2], D. F. CECHETTO[1], S. N. WHITEHEAD[1,3];
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Abstract: The aging brain is often characterized by the presence of pathological comorbidities resulting in accelerated brain deterioration. This has been demonstrated through the interaction of Alzheimer's disease (AD) and stroke. Gangliosides, a family of membrane lipids enriched in the central nervous system, are heterogeneously expressed and maintain a homeostatic distribution in the healthy mammalian brain. Alterations in their homeostatic expression profiles have been linked to a number of neurodegenerative disease and injury states including AD and stroke but has yet to be explored in the context of comorbidity. The accumulation of simple ganglioside species such as GM3 has been shown to induce degeneration and apoptosis and may thus play a mechanistic role in the brain's response to injury. This study employs Matrix-Assisted Laser Desorption Ionization (MALDI) Imaging Mass Spectrometry (IMS), a novel molecular imaging technique, to study the expression of A-series ganglioside species GD1a, GM1, GM2, and GM3 to determine how their expression profiles were altered in the presence of beta-amyloid (Aβ) toxicity in addition to ischemic injury. 3 month old male Wistar rats were given either a unilateral striatal injection of endothelin-1 (stroke group), bilateral intracerebralventricular (icv) injections of Aβ (25-35) (Aβ toxicity group), a striatal injection of endothelin-1 and icv injections of Aβ (25-35) (combined group), or underwent sham surgery. A significant increase in the simple ganglioside species GM2 was observed 3 days after surgery in the stroke group, while both GM2 and GM3 were significantly elevated in the combined group. By 21 d after surgery, GM3 was elevated in the stroke group while GM2 returned to control levels. In the combined group, both GM2 and GM3 remained elevated at 21 d. The observed accumulation and persistent expression of simple ganglioside species GM2 and GM3 in the combined group may be indicative of a mechanism of increased interaction between AD and ischemic injury.

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Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.06/DD10

Topic: C.21.Stroke Recovery

Support: EU FP7 grant 2780006 "NeuroFGL"

KoelnFortune Program, University of Cologne

Title: In-vivo analysis of brain-resident and blood-derived inflammation after experimental stroke

Authors: *M. SCHROETER[1], H. L. WALTER[1], M. WALBERER[2], M. A. RUEGER[1], H. BACKES[3], D. WIEDERMANN[3], M. HOEHN[3], B. NEURMAIER[3], R. GRAF[3], G. R. FINK[4]; [1]Dept. of Neurology, Univ. Hosp. Cologne, Cologne, Germany; [2]Univ. Hosp. Cologne, Cologne, Germany; [3]Max-Planck-Institute for Neurolog. Res., Cologne, Germany; [4]Res. Ctr. Juelich, Juelich, Germany

Abstract: In-vivo imaging of inflammatory processes induced by cerebral ischemia is a valuable tool to assess new therapeutical approaches in stroke research. We here evaluated the combination of two imaging modalities: magnetic resonance imaging (MRI) using intravenously applied ultra small supraparamagnetic iron oxide particles (USPIO), and positron emission tomography (PET) with the tracer [11C]PK11195. To specifically address the chronic phase after stroke, rats subjected to permanent middle cerebral artery occlusion (pMCAO) were followed for 56 days after induction of ischemia. Haematogenous phagocytes with internalized USPIOs induced MRI-T2* signal alterations after invading the brain. Combined analysis with [11C]PK11195-accumulation allowed to differentiate between brain-resident (intrinsic) and blood-derived (extrinsic) fractions of inflammation. Further quantification revealed an early intrinsically dominated inflammatory response preceding an increasingly haematogenous inflammatory reaction in the later phases. Tissue affected by intrinsic inflammation mostly remained in a vital but remodelled state, while extrinsic inflammatory responses were associated with severe injury and necrosis. Immunohistochemical endpoint analysis at day 28 or day 56 confirmed colocalization of Iba1+ activated microglia with [11C]PK11195 and iron as well as ED1/CD68 with USPIOs. However, naturally deposited iron could only be distinguished from USPIO-related iron accumulation by assessing MRI before and after USPIO application. We conclude that the combined approach using USPIO-MRI and [11C]PK11195-PET allows to

dissect post-stroke inflammatory processes in the living animal in an intraindividual and longitudinal fashion, and to predict individual tissue outcome.

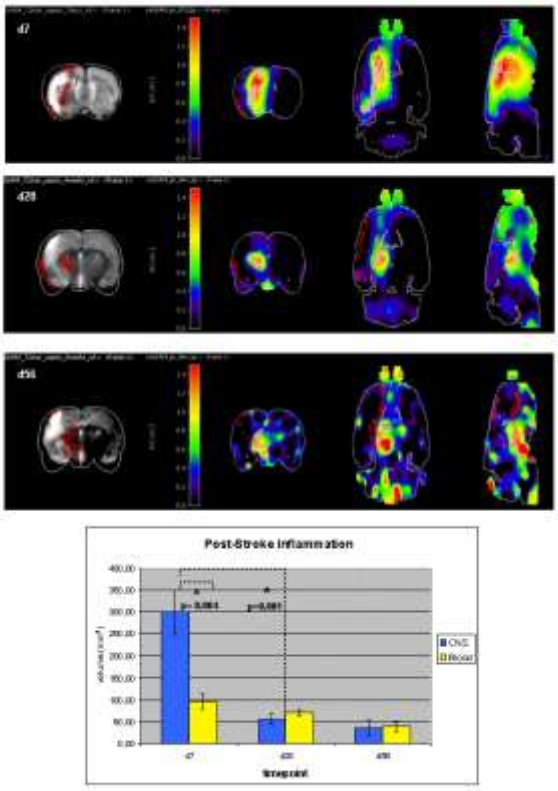

Fig. Dynamics of USPIO Enhancement (left column, red VOIs) superposed to PK11195 accumulation in an exemplaric animal at day 7, 28, and 56 after stroke. Quantification of USPIO+ and PK11195+ volumes (n=5) indicates the predominance of resident microglia at day 7, and of hematogeneous cells thereafter.

Disclosures: **M. Schroeter:** 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.; GlaxoSmithCline. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); Astellas Pharma, Bayer Healthcare, Biogen Idec, GlaxoSmithKline, Merck Serono, Pfizer, Roche, Sanofi Aventis, Talecris, Teva. **H.L. Walter:** None. **M. Walberer:** None. **M.A. Rueger:** None. **H. Backes:** None. **D. Wiedermann:** None. **M. Hoehn:** None. **B. Neurmaier:** None. **R. Graf:** None. **G.R. Fink:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.07/DD11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Neural mechanisms of performance monitoring during a physical exertion task in Chronic Fatigue Syndrome

Authors: *M. E. VAN DER SCHAAF[1,2], I. TONI[1], K. ROELOFS[1,3], F. DE LANGE[1], D. GEURTS[1], J. VAN DER MEER[2], H. KNOOP[2];
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Abstract: Chronic fatigue syndrome (CFS) is characterized by profound disabling fatigue without a known somatic explanation. Based on previous neuroanatomical and functional magnetic resonance imaging (fMRI) findings, it has been suggested that central neural mechanisms may underlie CFS symptoms. Here we have used fMRI to identify neural correlates underlying CFS symptoms. CFS is often associated with reduced performance on physical exertion tasks that cannot be explained by a lack of fitness or ability to deliver muscle force. Clinical work demonstrates that patients have dysfunctional beliefs about their ability to be active. Successful treatment with cognitive behavioural therapy is mediated by a change in these beliefs (Knoop et al, 2010). Combining this evidence with a model of Bayesian hierarchical inference (Edwards et al, 2012), we hypothesize that physical under-performance may arise from prior beliefs of being unable to adequately perform a physically demanding task. Crucially, this model leads to the prediction that those prior beliefs affect sensory perception and feedback processing, leading to reduced behavioural and neural adaptations when task feedback indicates that too little physical effort has been produced. To test this hypothesis we have investigated neural mechanisms involved in performance monitoring during a physical exertion task in female CFS patients diagnosed according to CDC-criteria and in age-, gender-, and education-matched healthy controls. In the task, participants were visually instructed to prepare and then squeeze a force-transducer at 30, 50 and 70% of their maximal voluntary contraction ability. After a jittered interval (500-3500 msec), subjects were informed about whether they squeezed too much, too little, or correctly. Preliminary analysis across 13 patients and 4 healthy controls showed that fatigue levels increased during the task. fMRI results revealed the involvement of motor and somatosensory areas during motor preparation/execution and of performance-monitoring areas following delivery of feedback. Consistent with the proposed hypothesis, the ventral anterior cingulate cortex of CFS patients showed differential feedback-related activity when feedback indicating the need to increase effort was compared to feedback indicating the need to reduce

effort. The study is ongoing and results of larger groups of CFS patients and healthy controls will be presented. We expect that these results will confirm that the feedback bias is specific for CFS patients, supporting the hypothesis that CFS patients have dysfunctional prior beliefs.

Disclosures: **M.E. Van Der Schaaf:** None. **I. Toni:** None. **K. Roelofs:** None. **F. De Lange:** None. **D. Geurts:** None. **J. van der Meer:** None. **H. Knoop:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.08/DD12

Topic: C.21.Stroke Recovery

Support: Stroke Association

Title: Heightened perception of effort after stroke: Evidence of increased activity in SMA and striatum

Authors: ***A. KUPPUSWAMY**, E. CLARK, I. TURNER, K. SANDHU, J. ROTHWELL, N. WARD;
Inst. of Neurology, UCL, London, United Kingdom

Abstract: After a stroke, many patients complain of daily activities requiring more effort, especially those who suffer from persistent fatigue. Effort, in the context of physical activities, is the subjective sense of muscular force that is thought to arise from central components that drive muscle contraction, possibly from secondary motor and sub-cortical structures. Any increase in the perception of effort may result from changes of activity in regions that drive motor output. Twelve chronic stroke patients with little motor weakness and no depression participated in a cross sectional observational study. Participants underwent functional magnetic resonance imaging whilst performing repetitive isometric hand grip using their affected hand. For each subject we characterised two orthogonal parameters, B(G) (average task related activity for all hand grips) and B(F) (the degree to which task related activity co-varied with peak grip force). Outside the scanner, participants performed an isometric biceps hold task. Two measures were collected during this task. A 1-10 numerical rating scale was used to measure perceived effort and transcranial magnetic stimulation was used to quantify central activation failure (CAF), a measure of excitability of inputs that drive motor output. We found that B(F) in SMA (0, 17, 46) was greater with increase in perception of effort (rsq=0.865, $p<0.001$). We also found B(F) in

striatum, bilaterally (18, -25, 19 & -18, -16, 19) was greater in those who have higher Central Activation Failure (rsq=0.79, rsq=0.804, p<0.001). Increased SMA activity is seen in those who perceive a given task to be more effortful. SMA has been implicated in a variety of functions associated with self-generated movement including processing of motor efference copy information. It maybe that the sense of effort arises from motor efference processing in the SMA. Central activation failure, a measure of how efficiently the motor output is being driven by input from rest of the brain, is seen to correlate with activity within the striatum, an important part of the cortico-subcortical motor loop. In a previous study we showed that CAF was significantly lower in those with low perceived effort suggesting that the activity within sub-cortical regions may also contribute to sense of effort. The results of this and previous studies taken together seem to suggest that alterations in effort perception after stroke arise from a distributed network of regions within the cortico-subcortical motor loop that encode effort.

Disclosures: **A. Kuppuswamy:** None. **E. Clark:** None. **I. Turner:** None. **K. Sandhu:** None. **J. Rothwell:** None. **N. Ward:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.09/DD13

Topic: C.21.Stroke Recovery

Support: RO1 NS056839

RO1 NS078791

R01 EB0011556

Title: Restoration of cortical blood flow precedes spontaneous forelimb recovery after cortical infarcts in mice

Authors: ***D. WOODIE**[1], S. KAZMI[2], M. FU[4], A. TANG[3], A. K. DUNN[2], T. A. JONES[1]; [1]Dept. of Psychology, [2]Dept. of Biomed. Engin., [3]Dept. of Biol., The Univ. of Texas, Austin, TX; [4]Neurobio., Duke Univ., Durham, NC

Abstract: There is typically some degree of spontaneous recovery over time after stroke and neural plasticity in the peri-infarct tissue has been implicated in this recovery. The temporal relationship between restoration of blood flow to peri-infarct tissue and spontaneous recovery

has not yet been investigated. We studied this in a mouse model of post-stroke upper extremity impairments by repeated within-animal measures of blood flow changes in peri-infarct cortex, as assayed with multi-exposure speckle imaging (MESI), and forelimb functional changes, as assayed with the pasta matrix reaching task (PMRT). Fourteen C57/BL6 mice were first shaped to determine their preferred-for-reaching forelimb on the PMRT. Cranial windows were then installed over the forelimb area of the contralateral motor cortex. After 3 weeks of recovery from cranial window surgery, the mice then received 3 baseline imaging sessions, followed by 14 days of training on the PMRT to establish pre-injury skill. Mice then received photothrombotic cortical lesions of the forelimb representation of the trained motor cortex or sham procedures (n's = 7). Mice received forelimb probes using the PMRT in tandem with the imaging of cortical blood flow at Days 3, 5, 10, and 20 post-infarct. Infarcts significantly decreased performance on the PMRT and reduced cortical blood flow compared to both baseline levels and the sham group. Additionally, the re-establishment of cortical blood flow proximal to the infarct core preceded the recovery of motor performance. The temporal patterns of results are consistent with the possibility that blood flow recovery enables the adaptive plasticity required for motor recovery.

Disclosures: D. Woodie: None. **S. Kazmi:** None. **M. Fu:** None. **A. Tang:** None. **A.K. Dunn:** None. **T.A. Jones:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.10/DD14

Topic: C.21.Stroke Recovery

Support: NIH R01DC005375

Title: Longitudinal recovery of naming in stroke patients: Preliminary findings

Authors: *R. SEBASTIAN[1], S. JARSO[2], J. PURCELL[1], C. DAVIS[1], Y. GOMEZ[1], J. CRINION[3], A. HILLIS[1];
[1]Neurol., [2]Radiology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom

Abstract: Introduction The neural mechanisms that support aphasia recovery are not yet fully understood. A previous, longitudinal fMRI study of auditory comprehension in stroke patients found that aphasia recovery is characterized by: little activation during the acute phase; a large

increase in activation in the bilateral network; and a shift of language back to the left hemisphere in the chronic phase (Saur et al., 2006). However, it is unclear whether other language tasks would show a similar time course in recovery and whether there would be individual differences in the recovery pattern. Our aim was to investigate the neural correlates of naming recovery from the acute to chronic stage. **Methods** 4 right-handed participants with acute ischemic left hemisphere stroke (mean age: 52.2 years) were enrolled. All participants received detailed language testing and scanning (diffusion weighted imaging, high-resolution structural and fMRI) at 3 time points (acute, 2-5 weeks and 6 months). The fMRI task was a cued picture-naming task. Each picture was presented concurrently with an auditory cue, which was either: (i) a whole word (ii) an initial phoneme or (iii) an unintelligible auditory noise. The control condition consisted of viewing scrambled pictures. Trials were presented in short blocks of six pictures, separated by the control condition of 7 s. fMRI data were preprocessed and analyzed in FSL 6.0. **Results and Discussion** All patients showed bilateral activation at the acute time point (Figure 1). Follow up scanning for Patient 3 did not show any significant change from the acute stage, although her naming ability had markedly improved, where as patient 4's naming ability continued to improve at the follow up time points with a corresponding increase in left hemisphere activation. Our preliminary data confirm that recovery of naming is dynamic, and show that there may be different time courses across tasks as well as across individuals. Further, brain reorganization during language recovery may not always proceed in three phases as proposed by Saur and colleagues.

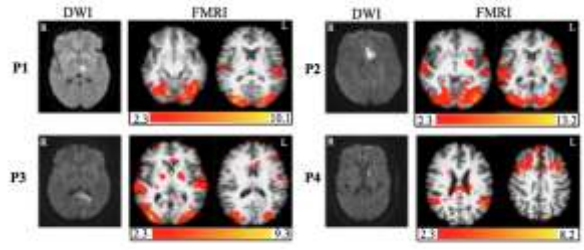


Figure 1: Diffusion Weighted Imaging (left) and FMRI activation maps (right) for the 4 participants at the acute time point (3days). DWI sequence shows an area of diffusion restriction in the left thalamus for Participant 1, left mesial frontal for participant 2, left PCA territory involving the parieto-occipital, parahippocampal and hippocampal region for participant 3, and left thalamus for participant 4 . FMRI data registered in MNI space shows areas of activation associated with correct picture naming (initial+ word cues) compared to viewing scrambled images at Day 3. Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of P=0.05.

Disclosures: R. Sebastian: None. **S. Jarso:** None. **J. Purcell:** None. **C. Davis:** None. **Y. Gomez:** None. **J. Crinion:** None. **A. Hillis:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.11/DD15

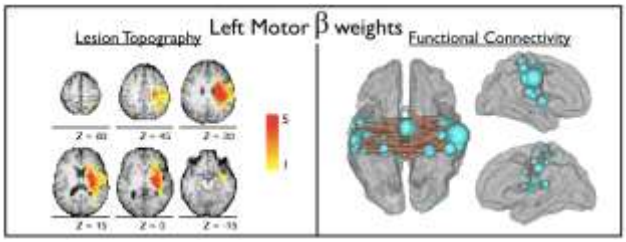
Topic: C.21.Stroke Recovery

Support: NIH Grant 5R01HD061117-08

Title: Lesion topography and functional connectivity disruption influence different domains of post-stroke deficit

Authors: *J. S. SIEGEL[1], L. E. RAMSEY[1], A. Z. SNYDER[2], R. V. CHACKO[3], K. Q. WEINBERGER[5], G. L. SHULMAN[1], M. CORBETTA[4];
[1]Neurol., [2]Neurology, Radiology, [3]Neurosurg., [4]Neurology, Radiology, Anat. & Neurobio., Washington Univ. Sch. of Med., Saint Louis, MO; [5]Computer Sci., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Abstract Deficits in stoke patients have classically been attributed to focal damage to particular parts of the brain (eg. expressive language, Broca's area). More recent evidence suggests that functionality is represented in widely distributed networks. We asked whether domain specific deficit (motor, language, spatial attention, spatial memory) is better predicted by lesion location or by functional disruption to specific networks. We recruited a heterogeneous cohort of 132 patients within 1-2 weeks following a first-time stroke. We acquired MRI, 35 min of resting state functional connectivity fMRI (FC), and an extensive behavioral battery spanning aforementioned domains. Lesions were manually segmented on the structural scans and FC correlations were calculated for each pair of 169 predefined ROIs. These measures of anatomical damage and FC correlations were entered into a leave-one-out ridge regression model to determine the percent of variance that could be explained in each behavioral domain. We mapped the most predictive weights back on to the cortex to visualize the locations and connections that are relevant to each of the domains tested (see figure). We found that some deficits corresponded better to lesion location while others corresponded better to long-range functional connections. Left motor deficit was better predicted by lesion location than FC (FC=31.7%, lesion=54.4% p=0.0045), language deficit was better predicted by lesion location than FC (FC=26.0%, lesion=44.2%, p=0.035), spatial memory deficit was better predicted by FC than lesion location (FC=38.5%, lesion =14.0%, p=0.0042), visual field neglect did not show a significant difference in predictive power of FC and lesion location (FC=31.4%, lesion =20.4%, p=0.3875). These results suggest that the relative merits of the localizationist view versus network view may depend on the domain of brain function.



Disclosures: **J.S. Siegel:** None. **L.E. Ramsey:** None. **A.Z. Snyder:** None. **R.V. Chacko:** None. **K.Q. Weinberger:** None. **G.L. Shulman:** None. **M. Corbetta:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.12/DD16

Topic: C.21.Stroke Recovery

Support: Heart and Stroke Foundation of Canada

Canadian Partnership for Stroke Recovery

Title: Impairments in cognitive function and resting network connectivity in chronic stroke

Authors: *A. M. AURIAT[1], J. K. FERRIS[2], L. A. BOYD[1];
[1]Physical Therapy, [2]Neurosci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Background Although rehabilitation research tends to focus on motor function it is clear that cognition has a critical role in determining quality of life after stroke. Unlike motor deficits, which are often predicted from size and location of injury, cognitive deficits are more difficult to relate to specific lesions. Resting state functional MRI (rfMRI) allows the study of brain networks in the absence of task performance and can identify connectivity abnormalities. Independent component analysis (ICA) of rFMRI does not require a priori definition of a seed region, allowing unbiased assessment of resting state networks. The current study assesses cognition and resting network activity, as identified by ICA, in high functioning chronic stroke participants. Methods All participants were living independently in the community. Neuropsychological assessment and magnetic resonance imaging, including several structural scans and 8 minutes of rfMRI, was obtained from 5 controls and 10 participants with chronic stroke. Cognitive assessments were compared between stroke participants and controls in a

multivariate analysis; results are reported as mean ± SD. Standard preprocessing of rfMRI was completed in SPM 8. ICA was completed using SPM 8 based Group ICA toolbox (GIFT). Previously identified network templates were used to classify activation in the mean composite activity maps of the stroke and healthy control groups. Results The suggested cutoff for mild cognitive impairment with the Montreal Cognitive Assessment is < 26. All controls scored ≥ 27, where as only 3 of our participants with stroke scored ≥ 26 (stroke, control: 22.4 ± 5.40, 28.8 ± 1.3). Stroke participants were significantly impaired compared to controls on several cognitive components including memory (California Verbal Learning Test, $p = 0.004$; 5.5 ± 1.1, 12.4 ± 1.6), attention/processing speed (Symbol Search, $p < 0.001$; 19.4 ± 8.8, 41.6 ± 7.4), and visuo-construction (Blocks, $p = 0.005$; 22.8 ± 13.0, 45.4 ± 9). However, there was no significant impairment in executive function (Clox, $p = 0.291$; 11.7 ± 3.7, 13.6 ± 1.1). The default mode network (DMN) was identified in both the stroke and healthy control groups. Compared to healthy controls the stroke group had less activation in hippocampus and posterior cingulate. Conclusions In a relatively high functioning group of chronic stroke participants (i.e. independent community dwelling) several components of cognition are impaired. Decreased connectivity in DMN may contribute to the cognitive deficits. Future analyses will examine the relationship between resting network activity, cognitive outcome and lesion size/location.

Disclosures: **A.M. Auriat:** None. **J.K. Ferris:** None. **L.A. Boyd:** None.

Poster

717. Stroke: Imaging and Diagnostics I

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Program#/Poster#: 717.13/DD17

Topic: C.21.Stroke Recovery

Support: NIH Grant 1K01HD069504

AHA Grant 13BGIA17120055

Title: Putting transcranial magnetic stimulation, diffusion tensor imaging and functional MRI to the test: A study of interhemispheric imbalance in chronic stroke

Authors: *D. A. CUNNINGHAM[1], A. MACHADO[2], D. JANINI[1], N. VARNERIN[1], C. BONNETT[1], S. ROELLE[1], G. YUE[4], S. JONES[3], M. LOWE[3], E. BEALL[3], K. SAKAIE[3], E. PLOW[1];

[1]Biomed. Engin., [2]Ctr. for Neurolog. Restoration, [3]Imaging Inst., Cleveland Clin., Cleveland, OH; [4]Human Performance & Engin. Lab., Kessler Fndn., West Orange, NJ

Abstract: It is believed hand deficits persist in stroke due to an imbalance between ipsilesional and contralesional hemispheres. Imbalance has been characterized in a few different ways: output of the corticospinal tracts as well as their integrity using transcranial magnetic stimulation (TMS) and diffusion tensor imaging, mutual transcallosal inhibition studied using TMS, and cortical activation in movement of paretic hand noted with functional MRI (fMRI). However, it is unclear whether different substrates describing imbalance even offer complementary perspectives, and how they subtend clinical function. Across ten stroke patients (63±9 years) with chronic upper-limb paresis, we examined associations between substrates of hemispheric imbalance, and their relation to two widely used outcomes- one measuring impairment and the other perceived disability in voluntary use of paretic hand. We have found that patients with poorer integrity of corticospinal tracts in the ipsilesional hemisphere show greater output of these tracts in the contralesional. However, neither an imbalance in their integrity nor an imbalance of their output relates to transcallosal inhibition. As a converse, imbalance in cortical activation was associated with transcallosal inhibition. Patients with relatively high fMRI activation within ipsilesional than contralesional motor cortices not only possessed stronger ipsilesional corticospinal output, but also showed balance of mutual transcallosal inhibition. Clinically, while patients with poorer integrity of corticospinal tracts in the ipsilesional hemisphere showed greater impairments, those with reduced ipsilesional than contralesional cortical activation had greater perception of disability. In conclusion, although output of contralesional corticospinal tracts and ipsilesional damage relates, mutual callosal influence is only associated with relative cortical activation between hemispheres. Still, viability of corticospinal tracts appear useful in categorizing range of specific impairments, and helping realize potential offered by the contralesional hemisphere in recovery, while fMRI activation serves to mark disability in volitional use of the weak hand.

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Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.14/DD18

Topic: C.21.Stroke Recovery

Support: ERC Advanced Grant 295129 (G.D. and M.H.A.)

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Leenaards Foundation (P.H.)

R01HD061117-08 (M.C.)

NIMH Fellowship 1F30MH099877-01-A1 (C.D.H.)

Title: Network modeling of resting state fMRI of stroke patients yields effective measures that correlate with behavioral performance impairments

Authors: *M. H. ADHIKARI[1], C. D. HACKER[2], A. GRIFFA[3,4], P. HAGMANN[3,4], G. DECO[1], M. CORBETTA[2];
[1]Ctr. for Brain and Cognition, Univ. of Pompeu Fabra, Barcelona, Spain; [2]Sch. of Medicine, Dept. of Neurology, Radiology, Anat. and Neurobio., Washington Univ. at St Louis, Saint Louis, MO; [3]Signal Processing Lab., Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [4]Dept. of Radiology and Lausanne Univ. Hosp., Univ. of Lausanne, Lausanne, Switzerland

Abstract: Mathematical modeling has been an effective tool in understanding the resting state activity of healthy brain but has rarely been used in case of patients suffering with stroke. We sought modeling based measures which could quantify the impact of stroke. We used the resting state functional connectivity (FC) from 19 healthy subjects and 24 stroke patients who had suffered cortical lesions during an acute phase after the occurrence of stroke. FCs were parcellated in 68 cortical areas and the percentage of damaged voxels in every parcel was found. We employed a mean-field reduction of a detailed realistic network model, consisting of excitatory and inhibitory populations of neurons, for each area and coupled the average activity of all excitatory populations using structural connectivity (SC) information obtained using diffusion spectrum imaging and a free parameter. We then simulated the model for several values of the free parameter to obtain a regime where the correlation between the simulated and empirical FC, for each stroke patient and an average healthy adult, was a maximum. Next, at this optimal working point, we calculated two measures: an integrative measure found by integrating the size of the largest connected component of the optimal simulated FC over a range of thresholds, and, a segregative entropy measure of simulated, evoked activity patterns in response to 1000 repetitions of task-like stimulations of a fraction of randomly chosen brain areas. These measures are representatives of the ability of the subject's brain in the resting-state for functional

integration and encoding distinct task-like stimuli respectively. We found that while the segregative ability of stroke patients was significantly less than that of an average healthy adult, values of integrative measures were greater. Further, we found a significant positive correlation between values of segregative measure for stroke patients and those of a composite measure of performance on behavioral tasks such as language and motor. Our results demonstrate that computational modeling based measures can be effectively used to characterize stroke induced impairment of the subject as well as to identify markers of recovery.

Disclosures: **M.H. Adhikari:** None. **C.D. Hacker:** None. **A. Griffa:** None. **P. Hagmann:** None. **G. Deco:** None. **M. Corbetta:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.15/DD19

Topic: C.21.Stroke Recovery

Support: NICHD Grant R01HD075740

Title: Plasticity in human motor system induced by somatosensory training in stroke patients

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Abstract: Residual somatosensory function is necessary for the re-acquisition of motor skills following stroke, and it is hypothesized that sensory recovery may precede and trigger improvements in motor function. Recently, we have shown that in healthy adults somatosensory perceptual learning can facilitate and accelerate motor learning by inducing changes in motor brain networks that are linearly independent of activity in somatosensory areas of the brain. Here, we have examined the effects of somatosensory training on brain motor networks and motor function in chronic stroke patients. We used a recently developed sensory perceptual training task in which vision of the limb was blocked, while patients (subcortical stroke, 2-6 years post-stroke) were required to judge whether their affected hand had been moved to the right or the left of the body midline. Patients held the handle of a robotic device that passively moved the arm outward in a horizontal plane on one of a set of fan-shaped paths. Binary feedback on the correctness of their responses was provided. We studied neural substrates of

perceptual training using fMRI to measure short-term changes in the brain's resting-state networks. Patients underwent scans of the resting brain before and after somatosensory training. Before the first scanning session and following the second one, subjects performed reaching movements in the absence of load. We used our shared and specific independent component analysis method to systematically identify brain networks whose functional connectivity was altered as a result of perceptual learning. In work to date, we find that somatosensory training increases functional connectivity in all patients in a sensory network including bilateral thalamus, striatum, cerebellum and ipsilesional Broca's area. It also strengthens functional connectivity in a motor-related network including ipsilesional dorsolateral premotor cortex, supplementary motor area and primary sensorimotor cortex, only in the well-recovered patient (Fugl-Meyer upper extremity score: 60/66). This network was partially activated following sensory training in the moderately recovered patient (FMA-UE 45/66), and was not activated at all in patient with severe stroke (FMA-UE 0/66). Likewise, in behavioral terms, only the well-recovered patient showed a transfer of sensory training to motor performance as quantified by deviations in reaching trajectories in active movements with no load. Overall, our results suggest that somatosensory training may aid in the recovery of not only sensory, but also motor function post-stroke by inducing adaptive plasticity in the ipsilesional sensorimotor cortex.

Disclosures: S. Vahdat: None. **M. Darainy:** None. **A. Thiel:** None. **D. Ostry:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.16/DD20

Topic: C.21.Stroke Recovery

Support: NINDS grant 5R01HD061117-08

Title: Disruption of interregional influence between Dorsal Attention Network and Visual Occipital Cortex following right hemisphere stroke

Authors: T. MEEHAN[1], *S. L. BRESSLER[2], S. V. ASTAFIEV[3], M. CORBETTA[3], G. L. SHULMAN[3];
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Abstract: Unilateral spatial neglect is a disabling syndrome in which, following predominantly right hemispheric focal lesions, the ability to attend to and consciously perceive events in contralesional space is decreased. Neglect can be caused by damage to a number of different regions, which may disrupt large-scale distributed networks that control attention [1]. One such network is the Dorsal Attention Network (DAN), which includes the frontal eye field (FEF) and intra-parietal sulcus (IPS). Evidence suggests that the DAN controls attention via top-down influences on the Visual Occipital Cortex (VOC) [2,3]. Such interregional influences can be measured by multivariate vector autoregressive modeling of Blood Oxygen Level-Dependent (BOLD) time series at the voxel-to-voxel level. We modeled influences between DAN and VOC regions from resting BOLD time series in three samples: subjects with neglect following right hemisphere damage (n=14), subjects with right hemisphere damage but no neglect (n=18), and healthy age-matched controls (n=32). In healthy age-matched controls, we demonstrate a resting asymmetry of influence between DAN and VOC regions, with top-down influences being greater than those in the bottom-up direction. We show that this asymmetry is disrupted in subjects with right hemisphere damage, which suggests that a physiological mechanism for attention may be disturbed in right hemisphere stroke patients. We are conducting further analyses to address confounds endemic to stroke vs. healthy contrast designs and to assess the relationship between disrupted network influences and the severity of neglect in patients. (Supported by NINDS grant 5R01HD061117-08) 1. Corbetta M, Shulman GL (2011) Annu Rev Neurosci, 34(1):569-599. 2. Tang W, Bressler SL, Sylvester CM, Shulman GL, Corbetta M (2012) PLoS Comp Biol, in press. 3. Bressler SL, Tang W, Sylvester CM, Shulman GL, Corbetta M (2008) J Neurosci, 28:10056-61.

Disclosures: T. Meehan: None. **S.L. Bressler:** None. **S.V. Astafiev:** None. **M. Corbetta:** None. **G.L. Shulman:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.17/DD21

Topic: C.21.Stroke Recovery

Support: KAKENHI Grant 24300147

Title: Dynamic neurite change responsible for motor recovery

Authors: *T. HAYASHI[1], N. HIGO[2], H. ZHANG[3], T. OSE[4], T. YAMAMOTO[5], Y. MURATA[2], H. ONOE[4];
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Abstract: Motor recovery after brain injury or stroke is thought to be achieved by reorganization of cortical and subcortical network of the residual brain. However, spatio-temporal dynamics of this network reorganization is not fully understood. In animal models of brain injury with motor recovery, we assessed long-term neuritic changes in the brain by evaluating coherence of dendritic and axonal orientation. Three rhesus monkeys were used for modelling primary motor (M1) cortical injury by injecting a neurotoxin, ibotenic acid, into the finger area of the right M1 cortex, which was identified by an intracortical microstimulation in each animal. Before and after the injury, their motor behavior was trained repeatedly every weekday and was assessed by a food-reach task in which animals learned to perform dextrous finger movements. Diffusion weighted magnetic resonance imaging (MRI) were also performed and the data was analyzed with models of diffusion tensor and neurite orientation dispersion for assessing local neurite architecture. Fractional anisotropy and neurite orientation dispersion were used for assessing neurite orientation coherence. Areas which were thought to be associated with motor recovery were injected tetrodotoxin (TTX) under a MRI-guided streotaxic system to confirm whether it actually re-disables motor function of the recovered hand. All the model animals showed total disability in dextrous finger movements of the left hand after M1 injury, followed by gradual improvement in success rate of the task in a period of three months. FA images depicted increases in neurite coherence in the ventral prefrontal white matter, ventral striatum, ventral premotor cortices, anterior intraparietal area and red nucleus, while decreased coherence was found in lesion-associate areas including hand area of the M1 and its connected areas. The increased neurite coherence was associated with degree of motor recovery, among which red nuclei and premotor white matter were responsible for recovered finger movements, as revealed by TTX inactivation. When an antero-grade neuronal tracer, biotinylated dextran amine, was injected into the ventral premotor area, positively labelled nerve terminals were found in the magnocellular part of red nucleus. The present study indicates that rewiring corticorubral output system contributes to the motor recovery after brain injury.

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Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

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Topic: C.21.Stroke Recovery

Support: This work was supported by NIH intramural research funds DC-000031-19 (to A.R. Braun).

Title: Neural correlates of story-comprehension performance in chronic aphasic patients

Authors: *S. LIU, M. HEALEY, S. BISSONNETTE, H. CHOW, Y. XU, M. PUERTOLAS LOPEZ, A. BRAUN;
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Abstract: Although real world communication exists predominantly at the discourse level, the current clinical assessment tools and therapies for aphasia are commonly limited to the word and sentence levels. Our lab identified a previously unrecognized population of aphasic patients who scored high on clinical tests of word and sentence level processing but still showed significant deficits at the discourse level. In an fMRI study of auditory story comprehension we explored the neural underpinnings of these residual discourse deficits, which have rarely been studied. We scanned 6 of these chronic patients (average 8.8 years post-stroke) and 7 age-matched controls. 12 naturalistic stories were used as stimuli, each having a complete narrative structure. Scrambled sentences generated from other matched stories were used to control semantic and syntactic complexity. Strings of Pseudo words with the same length were used to control for phonological complexity. Immediately after each story, 9 questions regarding story content were used to evaluate narrative comprehension. Group-level analyses showed similar activation patterns in controls and patients in both unlesioned left hemispheric language-related regions and right hemisphere homologues during sentence processing. This finding may explain why they scored high in clinical tests of sentenced processing. At the narrative level, the default mode network (DMN), which has been linked to discourse comprehension in control subjects, was also activated in patients. However, the anterior temporal and subcortical activations observed in controls were absent in patients. Significant decreases in posterior parietal cortices were observed in both groups. While group-level analysis revealed common activation patterns, individual-subject analysis was necessary in order to account for variability across patients. Patients' behavioral performance in story comprehension was used to determine the clinical relevance of both activations and deactivations. Results indicated that activation of the intact perisylvian areas was associated with good performance in a patient with a focal subcortical lesion. On the other hand, in patients with large temporal lesions activation of the DMN appears to play a significant compensatory role. In contrast, deactivations in posterior parietal cortices were found to interfere with the performance in all patients. These preliminary results reinforce the importance of studying aphasia at the discourse level, and reveal discrete brain areas that may be critical for recovery of natural language comprehension in post-stroke aphasic patients.

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Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.19/DD23

Topic: C.21.Stroke Recovery

Title: Leptomeningeal collateral circulation in acute stroke: Its relevance to the effectiveness of revascularization therapy

Authors: M. SUÁREZ-PINILLA[1], E. MORALES-DEZA[1], D. LARROSA-CAMPO[1], L. BENAVENTE-FERNÁNDEZ[1], *E. CARBONI[2], S. CALLEJA-PUERTA[1];
[1]Hosp. Universitario Central de Asturias, Oviedo, Spain; [2]Biomed. Sci., Univ. Cagliari, Cagliari, Italy

Abstract: Background: Leptomeningeal collateral circulation is deemed a pivotal factor in promoting survival of the endangered tissue during acute stroke, but there is little evidence on its relevance to the outcome of patients undergoing endovascular mechanical thrombectomy, a technique with a high recanalization rate in the first hours after the arterial occlusion. Methods: We prospectively recruited all acute stroke patients attended at our Hospital between 2012-13, in which a computed tomography angiography revealed a complete occlusion of an artery of the anterior cerebral circulation. Information on demographics, clinical and radiological features, treatments and outcomes was recorded. All CTA studies were separately reviewed by a neurologist and a radiologist. Leptomeningeal collateral circulation was assessed by comparison with the contralateral hemisphere, employing a semi-quantitative 4-point score (0=absent, 3=exhuberant). Results: Eighty-seven patients entered the study sample (63.2% male, median age 69). Collateral scores of 0,1,2 and 3 were given to 9%, 22%, 30% and 39%, respectively, with a negative association to age (p=0.002) and diabetes mellitus (p=0.039). Collateral score was found unrelated to the affected vessel, therapy choice and time from symptom onset. On the other hand, it showed an inverse association to the severity of the neurological deficit (NIHSS score) on admission (p=0.080) and at 24 (p=0.013) and 72 hours (p=0.042). On multivariable analyses, collateral score remained an independent predictor of outcome at 24h, 72h and at discharge as well as of the difference between the initial NIHSS score and the one at 24 and 72 hours. When considering only those patients undergoing mechanical thrombectomy, this

association remained for all the aforementioned endpoints even when recanalization success (TICI ≥ 2b) was included in the multivariable models ($p<0.010$ for all linear regression models). Clinical improvement at 72 hours was greater for those patients undergoing mechanical thrombectomy when collateralization was poor ($p=0.017$), but treatment choice did not significantly affect outcome in those with a good collateral score ($p=0.535$). Conclusions: Leptomeningeal collateral circulation shows a decisive prognostic value in acute stroke patients after adjustment for demographics, affected vessel, time from onset and therapy choice. It remains equally relevant for those patients undergoing endovascular mechanical thrombectomy, being an independent predictor of clinical improvement even after successful recanalization. Patients with poor collateralization benefit the most from this interventionist treatment.

Disclosures: **M. Suárez-Pinilla:** None. **E. Morales-Deza:** None. **D. Larrosa-Campo:** None. **L. Benavente-Fernández:** None. **E. Carboni:** None. **S. Calleja-Puerta:** None.

Poster

717. Stroke: Imaging and Diagnostics I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 717.20/DD24

Topic: C.21.Stroke Recovery

Support: NIH, NICHD, HD065438

Title: DTI analysis of corticospinal tract using BrainSuite: A potential biomarker of upper extremity therapeutic response to neurorehabilitation in chronic stroke

Authors: *B. KIM[1], D. B. KAY[1,2], Y. YI[1], D. LEE[1], Y. CHAUDHRY[1], J. P. HALDAR[3,4], R. M. LEAHY[3,4], C. J. WINSTEIN[1];
[1]Div. of Biokinesiology and Physical Therapy, [2]Neurosci. Grad. Program, [3]Ming Hsieh Dept. of Electrical Engin., [4]Brain and Creativity Inst., USC, Los Angeles, CA

Abstract: Corticospinal tract (CST) microstructural characteristics measured by diffusion tensor imaging (DTI) are known to be associated with upper extremity (UE) motor impairment after stroke. However, there is a gap in understanding the relationship between DTI-derived measures and UE motor function changes following neurorehabilitation. This study is part of a larger longitudinal phase-I clinical trial in chronic stroke that aims to determine the optimal dose of therapy for sustained affected arm use after therapy has ended. Our purpose is twofold: First, to establish methods to quantify CST characteristics in lesioned brains using BrainSuite. Second, to

determine if UE motor performance changes after treatment in chronic stroke are associated with DTI-based CST measures. Nine chronic stroke participants completed neuroimaging and clinical assessments before and after 12 sessions of a reproducible UE therapy program within 4 months. Imaging data were processed using BrainSuite (version 13a, http://brainsuite.org). Specifically, BrainSuite was used to semi-automatically extract and parcellate the participants' brains from T1-weighted structural MRI images, to correct the diffusion images for geometric distortion, to coregister the diffusion images with the structural images, to compute DTI parameters, to perform deterministic tractography, and to identify the CST based on the set of tracks that pass through both a manually labeled pons region of interest (ROI) and an automatically labeled precentral gyrus ROI. The ipsi- and contra-lesional CST fractional anisotropy (FA) was quantified, and the CST FA asymmetry index [(FAcontra - FAipsi)/(FAcontra + FAipsi)] was calculated. The Wolf motor function test (WMFT) and Fugl-Meyer assessment (FMA) were performed to assess participants' motor function and impairment respectively. Linear regression analysis was performed to examine the relationship between CST FA asymmetry and these clinical outcomes, based on the hypothesis that pre CST FA asymmetry would be correlated with the pre to post changes in the WMFT and FMA scores. Similar to previous reports, the pre CST FA asymmetry was positively correlated with the pre WMFT score. However, the pre and pre to post changes in CST FA asymmetry were not correlated with the pre FMA or pre to post changes in either FMA or WMFT scores. Because of the limited range of pre CST FA asymmetry indices for the nine participants in this study, a relationship between CST FA asymmetry and clinical outcomes could not be explained by our results. Thus, further investigations of DTI-derived measures in post-stroke individuals are necessary to identify biomarkers for functional recovery.

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Poster

717. Stroke: Imaging and Diagnostics I

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Topic: C.21.Stroke Recovery

Support: NIH Grant K01HD069504

AHA 13BGIA17120055

Title: Predicting variability of distal muscle recruitment curves in stroke using diffusion tensor imaging (DTI)

Authors: *K. A. POTTER-BAKER[1], N. M. VARNERIN[1], D. A. CUNNINGHAM[1], S. M. ROELLE[1], V. SANKARASUBRAMANIAN[1], A. MACHADO[1], A. B. CONFORTO[2], K. SAKAIE[1], E. B. PLOW[1];
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Abstract: Viability of corticospinal tracts (CST) has long been hypothesized to predict functional outcomes of upper limbs following lesions affecting the central nervous system, such as stroke or spinal cord injury. One highly utilized method for understanding CST viability, as a means of predicting and tracking longitudinal patient functional recovery, has been use of the non-invasive neurophysiologic technique transcranial magnetic stimulation (TMS). Specifically, within TMS, electromagnetic induction to the scalp overlying motor cortices (M1) results in an elicited contraction of the contralateral target muscle, termed motor evoked potentials (MEPs). In addition, MEP recruitment curves (RCs) are also determined by assessing MEP amplitudes at incremental stimulus intensities until a MEP plateau is reached. MEP amplitude and the area under the recruitment curve are believed to represent the gain or viability of descending CST to the target muscle and are utilized to track patient recovery. Unfortunately, however, within any patient population, MEPs are highly variable compromising measurement of RC at incremental intensities. Here, in a sample population of stroke patients we investigated whether the extreme variability of RCs can be ascribed to poor white matter integrity of in the CST, as measured by diffusion tensor imaging (DTI). As a metric of overall tract integrity, fractional anisotropy (FA) was compared between the lesioned and non-lesioned hemispheres for tracts projecting between the posterior limb of internal capsule (PLIC) and major cortices including: M1, premotor cortex (PMC) and supplementary motor area (SMA). FA was evaluated at 3 levels: (1) single-slice at the PLIC, (2) weighted average along the length of the tract and (3) segment of tract lying within the stroke/degenerated region. Notably, we found that variability in RCs was strongly correlated with segmental variations of FA for tracts converging from M1 ($R=-0.766$, $p<0.027$), PMC ($R=-0.647$, $p<0.083$) and SMA ($R=-0.673$, $p<0.067$). In addition, we found that RC variability was significantly predicted by segmental variation of FA along tracts emerging from M1 ($\beta=-0.766$, $p<0.027$). Collectively, our results suggest that of the three investigated types of DTI analysis, segmental FA values may provide the strongest clinical sensitivity in predicting variations in MEP generation in distal muscles. In addition, by reconstructing tracts to three major cortices, future work can now begin to predict recovery potential based on the viability of individual CSTs. Finally, by isolating potential sources for variability in of MEP measurement, we hope to further improve the diagnostic ability of TMS.

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Poster

718. Stroke: Imaging and Diagnostics II

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Topic: C.21.Stroke Recovery

Support: PHRC Grant 98/CHUG/15/C2

Title: Prediction of sensorimotor recovery in pure motor hemiparesis using fMRI

Authors: *T. ZEFFIRO[1], A. JAILLARD[2];
[1]Neural Systems Group, Neurometrika, POTOMAC, MD; [2]CHU Grenoble, Grenoble, France

Abstract: Purpose: Predicting which patients will have good recovery or an optimal therapeutic response following stroke is complex. Functional MRI (fMRI) provides efficient and non-invasive estimates of neural activity throughout the recovery period. A recent meta-analysis of fMRI studies demonstrated that ipsilesional primary motor cortex (MI) and supplementary motor (SMA) activity measured in the chronic stage are associated with good sensorimotor recovery after stroke involving the motor system (1). In this study we asked whether ipsilesional cortical activity measured soon after purely subcortical ischemic damage predicted later sensorimotor performance. Methods: We studied 18 stroke patients with pure motor hemiparesis after their first lacunar stroke. Lesions were limited to the deep territory of the anterior choroidal artery involving the corticospinal tract at the level of the internal capsule or corona radiate and caused pure limb weakness demonstrable on admission. Patients were matched to 18 healthy controls for age and sex. Motor impairment was assessed using the NIH Stroke Scale (NIHSS), the Fugl-Meyer scale (FMS), finger opposition speed, pegboard performance and sensorimotor reaction times collected one week and six months after stroke, allowing construction of a global motor recovery score. fMRI data were acquired using a self-paced finger opposition (FO) task alternating right and left FO with rest. At the group level, regression was used to assess the predictive utility of neural activity measures collected in the acute period on sensorimotor recovery measured at six months. Age and FO rate were included as covariates. Results: All patients showed good sensorimotor recovery at six months and both patients and controls exhibited a typical motor activity pattern during sequential finger opposition. Both ipsilesional MI and SMA activity in the acute period predicted motor recovery at 6 months ($p<0.001$), after

adjustment for age and movement rate. Conclusion: Ipsilesional sensorimotor cortical activity, measured soon after stroke onset, predicts motor recovery assessed six months later. With measurements made in the early phase of stroke recovery, fMRI could be used either in clinical practice or to acquire prognostic biomarkers in clinical trials investigating novel treatments, such as autologous stem cell administration. (1) Upper Limb Recovery After Stroke Is Associated With Ipsilesional Primary Motor Cortical Activity: A Meta-Analysis, Stroke 45:1077-1083 (2014)

Disclosures: T. Zeffiro: None. **A. Jaillard:** None.

Poster

718. Stroke: Imaging and Diagnostics II

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Topic: C.21.Stroke Recovery

Support: Veni Grant to Vivian Weerdesteyn

Title: Are delayed postural responses to perturbations associated with poorer balance capacity in people after stroke?

Authors: *D. DE KAM, A. BRUIJNES, J. ROELOFS, A. C. H. GEURTS, V. WEERDESTEYN;
Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: ***Objective***: Postural instability is among the most important risk factors for falls in people after stroke. Postural responses to balance perturbations are delayed in these patients [1,2]. We aimed to identify whether delayed responses are associated with poorer balance capacity in people in the chronic phase after stroke. ***Methods:*** Ten people after a unilateral stroke (>6 months) and nine healthy controls were subjected to translational balance perturbations in four random directions. Using an iterative protocol, we identified the highest perturbation intensity that could be recovered 1) without stepping (stepping threshold), and 2) with a maximum of one step (limit of stability). The protocol also included additional perturbations at intensities of 0.5 m/s^2 and 1.5 m/s^2 (n=4 for each direction and intensity) to allow between-subjects comparisons of muscle onset latencies. We determined the onset latencies of tibialis anterior and rectus femoris for backward perturbations; gastrocnemius, soleus and biceps femoris for forward perturbations; and the gluteus medius and peroneus on the perturbed side for lateral

perturbations. We compared onset latencies, stepping thresholds and limits of stability between patients and controls. Pearson correlations were calculated between onset latencies and stepping thresholds and limits of stability. ***Results:*** Irrespective of perturbation direction, people with stroke had lower limits of stability than controls ($p<0.05$). Stepping thresholds in patients were lower for forward perturbations ($p=0.01$), with a similar tendency for backward perturbations ($p=0.10$). Onset latencies at 0.5 m/s^2 were delayed in all the muscles of the affected leg. At 1.5 m/s^2 delays were smaller and no longer significant for the peroneus and calf muscles. Onset latencies in the unaffected leg were not different from controls. Longer muscle onset latencies in the affected leg were not associated with poorer balance recovery, except for a moderate correlation between tibialis anterior onset and backward stepping threshold ($R^2=0.44$, $p=0.04$). ***Conclusions:*** Delayed postural responses may contribute to impaired backward feet-in-place balance recovery responses, but they did not seem to underlie the poorer reactive stepping capacity in this small group of people after stroke. Interestingly, we observed that group differences in onset latencies became less prominent with increasing perturbation intensity, which may point at reduced sensitivity of postural circuits to proprioceptive input following stroke. ***References:*** 1. Marigold 2006, 2. Kirker 2000.

Disclosures: **D. De Kam:** A. Employment/Salary (full or part-time):; Radboud University Medical Center. **A. Bruijnes:** None. **J. Roelofs:** None. **A.C.H. Geurts:** None. **V. Weerdesteyn:** None.

Poster

718. Stroke: Imaging and Diagnostics II

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: C.21.Stroke Recovery

Support: VA CSR&D Merit Award CX000586

Title: Characterizing white matter disconnection in stroke with constrained spherical deconvolution tractography

Authors: *A. U. TURKEN;
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Abstract: Accurate characterization of white matter fiber pathway disconnection in brain injury is important for the diagnosis and treatment of neurological patients, as well as the analysis of

lesion-deficit correlations for gaining insights into white matter function and the effects of disconnecting lesions. Here, we used structural and diffusion MRI data from a group of stroke patients and demographically-matched controls in order to assess MRI-based white matter lesion analysis protocols. The study group included 18 chronic (> 6 months) stroke patients with unilateral lesions (MCA territory, 15 left, 3 right hemisphere). MRI brain images were acquired on a Siemens Magnetom 3T Verio scanner. We used structural images (T1, T2, FLAIR) to manually delineate lesions and to transform patient images to a standard space for atlas-based analysis of white matter tract damage (Catani and de Schotten, 2008). We used high angular resolution diffusion imaging (HARDI, b = 2000 s/mm2, 64 directions) data to assess white matter pathways with constrained spherical decomposition (CSD, Tournier et al., 2004) tractography. We delineated major tracts manually as well as with automated fiber clustering (Yeatman et al., 2012), and used the number of tractography-derived streamlines for each tract to quantify white matter fiber pathway disconnection in stroke patients relative to the control group. As accurate registration of brain structures to a standard template is critical for the atlas-based analysis of lesion data (Brett et al., 2001; Crinion et al., 2008), we also examined the impact of the spatial normalization protocol on white matter tract registration. We found that lesion reconstructions alone can underestimate white matter damage when tract degeneration outside the primary lesion is not quantified, and potentially overestimate white matter damage when tractography reveals surviving fibers in lesioned areas. We also found that standard spatial normalization protocols are not adequate for accurate white matter tract registration in patients with ventricular dilation. Overall, HARDI-based probabilistic tractography, combined with optimized spatial normalization, provides a significantly improved framework for white matter disconnection analysis in stroke.

Disclosures: **A.U. Turken:** A. Employment/Salary (full or part-time):; Veterans Affairs Northern California Health Care System.

Poster

718. Stroke: Imaging and Diagnostics II

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Topic: C.21.Stroke Recovery

Support: Heart and Stroke Foundation of Canada Research Fellowship to M Borich

Canadian Institutes of Health Research; MOP-106651

Title: Simultaneous TMS-EEG to assess transcallosal inhibition in chronic stroke

Authors: *M. R. BORICH[1], S. M. BRODIE[2], B. LAKHANI[2], L. A. BOYD[2];
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Abstract: Background Stroke is the leading cause of long-term adult disability with ~50% of stroke survivors living with arm dysfunction. It is hypothesized that down-regulated activity in the stroke-lesioned primary motor cortex (iM1) and up-regulated activity in the contralesional (cM1) may be principally responsible for this persisting dysfunction. These changes in hemispheric activity have been measured indirectly with techniques lacking sufficient spatial and temporal resolution. The purpose of this preliminary work is to utilize real-time integration of noninvasive brain stimulation (transcranial magnetic stimulation or TMS) and electroencephalography (EEG) to evaluate TMS-evoked cortical activity applied to either iM1 or cM1 in individuals with chronic stroke. Methods Nine individuals with chronic stroke (mean age±SD: 66±4 years, post-stroke duration: 67±47months, 9M/0F) and impaired arm function (Fugl-Meyer score: 40±23) were recruited. Standard TMS procedures were conducted to identify stimulation site and motor thresholds for the extensor carpi radialis muscle bilaterally. Transcallosal inhibition (TCI) was evaluated using single TMS pulses (20 trials, <0.2Hz, 1.5x resting motor threshold) over M1 while performing an ipsilateral grip force contraction (50% maximum). 64-channel EEG was collected during TCI with a TMS-compatible DC amplifier. EEG data were processed and analyzed in EEGLAB. Due to TMS artifact, EEG data were analyzed from 40-200ms post-TMS. TMS-evoked potentials (TEPs) were identified in the homotopic EEG channel contralateral to the site of stimulation (e.g. extract TEPs in C4 when stimulating near C3). Results Preliminary analyses demonstrate the ability to elicit measurable TEPs in either hemisphere regardless of stroke severity. Data suggest altered contralateral TEP characteristics elicited by iM1 stimulation compared to cM1. In general, iM1 stimulation showed a sustained positive deflection contralaterally from ~160-190ms post-TMS. Whereas, cM1 stimulation typically elicited a positive deflection contralaterally around 60-70ms post-TMS. Discussion These preliminary results are the first attempt at characterizing TEPs using real-time TMS-EEG integration in individuals with stroke. This technique offers the capacity to characterize the causal mechanisms underlying differences in brain excitability and connectivity in stroke. Differences in the temporal characteristics of contralateral TEPs may indicate differences in interhemispheric interactions after stroke. Thus, it may be possible to develop novel biomarkers of neural activity associated with recovery and treatment response in stroke.

Disclosures: **M.R. Borich:** None. **S.M. Brodie:** None. **B. Lakhani:** None. **L.A. Boyd:** None.

Poster

718. Stroke: Imaging and Diagnostics II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 718.05/DD30

Topic: C.21.Stroke Recovery

Support: EKFS Grant 2011_119

Title: Functional and structural connectivity of the red nucleus after stroke

Authors: R. LINDENBERG[1], D. WERDER[1], B. TAUD[1], J. BRECHT[1], M. M. SIEG[1], M. MEINZER[2], *A. FLOEL[1];
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Abstract: Studies on human and non-human primates demonstrated that the cortico-rubro-spinal system (CRSS) can compensate for damage to the pyramidal tract (PT). While previous studies revealed structural alterations of the CRSS as the result of plastic changes in the course of recovery, little is known about corresponding modifications of its functional connectivity. Combining diffusion tensor imaging (DTI) and resting state functional MRI (RS-fMRI), we aimed at exploring structural and functional connections of the red nucleus in a group of chronic stroke patients. 28 chronic stroke patients (7 women, mean age 60.5±11.6 yrs; >6 months after stroke; 17 right- and 11 left-hemisphere lesions) underwent MRI at 3T including DTI and RS-fMRI. Residual motor function of their affected arm was assessed with the Fugl-Meyer-Upper Extremity Score (UE-FM). According to previous work from our group, PT and CRSS were reconstructed using probabilistic tractography algorithms implemented in FSL. Voxel-wise correlation analyses of fractional anisotropy (FA) and UE-FM were conducted in both tracts of either hemisphere. Similar to previous studies, FA along ipsilesional PT and CRSS correlated with UE-FM (higher FA = better function), while a cluster in the vicinity of the ipsilesional red nucleus showed an inverse correlation (lower FA = better function; all results FDR corrected for multiple comparisons). No clusters were found in contralesional tracts. Using the red nucleus cluster and its contralateral counterpart as seeds, we performed whole brain correlation analyses of RS-fMRI. For comparisons of ipsilesional and contralesional hemispheres, we conducted paired t-tests of resulting maps. The ipsilesional red nucleus was significantly stronger connected with ipsilesional basal ganglia and pre-supplementary motor area, while the contralesional red nucleus maintained stronger connections with ipsilesional primary and cingulate motor areas as well as contralesional superior parietal cortex. In conclusion, we replicated previous findings of correlations between red nucleus microstructure and residual motor deficit after stroke in an independent group of patients. In addition, we found hemispheric differences in functional network architecture of areas connected with bilateral red nuclei. Most notably, connections between contralesional red nucleus and ipsilesional motor areas were strengthened. Those results

shed light on functional and structural reorganization of the motor system after stroke, which may help fostering the development of novel restorative treatments.

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Poster

718. Stroke: Imaging and Diagnostics II

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Program#/Poster#: 718.06/DD31

Topic: C.21.Stroke Recovery

Support: CIHR MOP-106651

Title: Altered motor planning network activity after stroke: Resting state methodology and analysis

Authors: *S. PETERS, M. J. MCKEOWN, J. GARLAND, B. LUU, L. A. BOYD;
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Abstract: Objective: Analyzing the pattern of network activity enables the study of potential widespread neural activity changes after a focal lesion. At rest, the function of motor planning is to sustain readiness for possible movements. The purpose of this study is to examine the motor planning network activity after stroke by comparing full and partial correlation analysis of resting state functional magnetic resonance imaging (fMRI). Methods: An anatomical T1 scan was completed on one post-stroke participant in a 3T MRI Philips scanner followed by functional MRI using BOLD contrast (flip angle=90degrees, TR=2s, TE=30ms, scan time=8.2min) with the subject lying quietly with eyes open and fixated on a standard image. Manual region of interest (ROI) drawing included: the bilateral premotor (PMc), motor (M1), and supplementary motor (SMA) cortices, cerebellum (Cb) and basal ganglia (BG) - regions known to be involved with motor planning. At each time point, the average of all voxels' BOLD signal within the ROI was calculated. Full correlation (FC) measures the degree that two or more variables fluctuate together where partial correlation (PC) removes the influence of all the other variables from the pair that is being tested, and then measures the degree of association remaining. To use PC, the analysis must have more time series data points than ROI's. FC and PC were performed with statistical significance set at $p<=0.001$. Results: FC and PC demonstrated different network activity. Despite FC providing many statistically significant

results, it did not identify an important connection between the ipsilesional PMc/M1 that PC uncovered ($r = 0.3118$, $p = 0.00000083$). Contralesionally in the PC analysis the SMA was connected with M1 ($r = 0.3130$, $p = 0.00000075$) while PMc was not. Conclusions: Without the connectivity between M1 and PMc on the contralesional side, it is possible that SMA functions in a compensatory way due to the lack of PMc input to M1. With the function of both SMA/PMc known to be involved with motor planning, it is possible that after a stroke, one region may increase its network activity to functionally offset the other's reduced network activity. In this analysis, both hemispheres indicate altered motor network activity, which may render this individual less ready for voluntary movement. After stroke, reduced network activity for motor planning was demonstrated at rest using both FC and PC approaches, but the motor network was more evident with PC. This case study provides an example of how two relatively simple network analysis methods can be used examine motor planning network activity after stroke.

Disclosures: S. Peters: None. **M.J. McKeown:** None. **J. Garland:** None. **B. Luu:** None. **L.A. Boyd:** None.

Poster

718. Stroke: Imaging and Diagnostics II

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Topic: C.21.Stroke Recovery

Support: NIH Grant R00 HD065839

Mable H. Flory Grant

Title: Neurally dissociable information-processing components of reading deficits in subacute stroke

Authors: *O. BOUKRINA[1], E. J. ALEXANDER[1], A. M. BARRETT[2,3], W. W. GRAVES[1]; [1]Psychology Dept., Rutgers, The State Univ. of New Jersey, Newark, NJ; [2]Stroke Rehabil. Res., Kessler Fndn., West Orange, NJ; [3]Physical Med. and Rehabil., Rutgers-New Jersey Med. Sch., Newark, NJ

Abstract: A prevailing cognitive model of reading proposes that words are processed by interacting orthographic (spelling), phonological (sound) and semantic (meaning) information. To identify the brain regions critical for carrying out this information processing, we have so far

performed neuropsychological testing and multi-sequence MRI on 5 patients with left-hemisphere stroke (<= 5 weeks post onset). We expected reading aloud to require all three components, while reading pronounceable letter strings (pseudowords, e.g., blork) can be completed without semantics. If a patient is able to read pseudowords, but not words, and especially words that do not follow typical spelling-sound patterns (e.g., yacht), this is described as surface dyslexia and is thought to arise primarily from semantic deficits. In contrast, if a patient has difficulty reading pseudowords aloud or making phonological judgments about them, this is described as phonological dyslexia and is thought to arise from deficits in mapping orthography to phonology. In our sample, two patients (P1,P3) were unimpaired across all three components, two patients (P2,P5) showed a pattern of phonological dyslexia, and one (P4) had a profound impairment in reading words and pseudowords. While lesion size predicted the overall degree of impairment, lesion location was crucial for predicting the type of impairment. In the two patients with phonological dyslexia, the damaged areas included the left corona radiata (CR)/internal capsule, with extension to thalamus and putamen in P5. P2, with a restricted CR stroke, achieved 83% accuracy in reading aloud words and only 33% accuracy for pseudowords, $t(178) = 8.51$, $p<.0001$. P5 also performed better on words (63%), than pseudowords (42%), $t(178) = 2.81$, $p<.01$. This is in contrast with similar performance on words and pseudowords in P1 and P4. P2 was relatively unimpaired on a semantic matching task (69%), but impaired on phonological (62%) and orthographic (67%) tasks compared to the two highest scoring patients. Relative to the other patients and a sample of 21 healthy controls, P2 showed exaggerated effects of word frequency, consistency, and their interaction. This is consistent with impaired orthography-phonology mapping and intact semantics. As we continue this study, we will evaluate whether spared deep fronto-parietal white matter connections to the thalamus are associated with intact orthographic-phonological mapping. Future studies will be useful investigating whether targeting reading therapies to impaired information processing components may be appropriate in the first weeks after stroke.

Disclosures: O. Boukrina: None. **E.J. Alexander:** None. **A.M. Barrett:** None. **W.W. Graves:** None.

Poster

718. Stroke: Imaging and Diagnostics II

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Program#/Poster#: 718.08/EE1

Topic: C.21.Stroke Recovery

Support: NIH Grant GM103503

Title: Pilot testing of a PET insert for MRI: Preliminary results and clinical and research applications

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Abstract: MRI is the dominant brain imaging modality currently employed in the neuroscience community due to its superior resolution, soft matter differentiation, and timing resolution in comparison to other imaging modalities. However, in essence, MRI is not truly a molecular imaging technique, as it currently lacks the ability to trace and quantify individual biomarkers in the human brain, at least beyond an experimental phase, and MR spectroscopy is very low resolution. Here, we demonstrate that a compact, economical PET insert can fit tightly around a participants's head in the scanner and provide images simultaneously with MRI. This finding shows that, in theory, compact economical PET inserts can be widely implemented and installed in any existing MRI scanner. This is because the insert which we used in this study uses non-specific RF receiving coils to enhance the MRI image as opposed to built-in RF elements or the widely available "birdcage" head coil. Methods: In this study, we conducted multiple experiments inside a 3 Tesla MRI to see how the magnetic field would influence the operation of our PET imager. We conducted simultaneous and sequential PET/MRI scanning of a Hoffman brain phantom with multiple pulse sequences. Results: it was shown that the quality of the MR image was largely unaffected by the PET imager. Furthermore, although the quality of PET imaging was affected by the RF pulsing, an acceptable PET image was nevertheless produced. Furthermore, sequential imaging showed high image quality in both modalities. Conclusion: This study shows that our PET insert is MR compatible, and that next generation devices based on its concepts will continue to improve combined PET/MRI functionality. Two of the major advantages of this imager are its adaptability and the potential for low-dose scanning, with detectors are close to the head so radioligand dose may be reduced to potentially 1/10 standard dose, plus using the structural MRI for attenuation would eliminate need for CT dose. The device would have potential for clinical applications such as acute stroke or other conditions requiring both MRI and PET, eliminating need for two separate scans and reducing dose. Research applications may include examining the relationship between metabolic or neurotransmitter distribution and fMRI, DTI, or rsMRI activation for various mental and meditation states, as well as basic research involving neurological disorders. Overall, this study suggests that PET/MRI brain imaging with low dose is possible using an insert which could be adapted to any MRI scanner, using standard RF coils.

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Poster

718. Stroke: Imaging and Diagnostics II

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Topic: C.21.Stroke Recovery

Support: Intramural Research Program, NINDS

Intramural Research Program, NIH Clinical Center

Title: Cortical activation and inter-hemispheric sensorimotor coherence in individuals with arm dystonia due to childhood stroke

Authors: *S. N. KUKKE[1], A. DE CAMPOS[2], D. DAMIANO[2], K. ALTER[2], M. HALLETT[2]; [1]Biomed. Engin., The Catholic Univ. of America, Washington, DC; [2]NIH, Bethesda, MD

Abstract: Motor disorders following childhood stroke are common, yet are insufficiently studied. Of the observed post-stroke motor sequelae, childhood dystonia is the most frequently occurring disorder. Post-stroke dystonia is most commonly observed on the side of the body contralateral to the stroke. Unilateral dystonia in this population usually affects the arm, prevents the development of typical arm function, and can lead to substantial disability persisting into adulthood. The purpose of this study was to explore the neurophysiological basis of arm dystonia due to childhood stroke, which is a critical step to developing and selecting effective treatments. In this observational case-control study, electroencephalography (EEG) was used to compare sensorimotor EEG spectral power (Pow) and inter-hemispheric sensorimotor coherence (IHCoh) between individuals with dystonia due to childhood stroke (n=7, 20+/-3 yrs) and healthy volunteers (n=9, 17+/-5 yrs) at rest and during isometric wrist extension. All patients had the diagnosis of unilateral cerebral palsy, which is a common outcome of early stroke with similar risk factors and associated motor control challenges. Results indicated abnormally low resting IHCoh in patients that correlated significantly with arm dystonia severity (Burke-Fahn-Marsden dystonia rating scale) and poor hand function (Manual Ability Classification System). Patients also had decreased task-related Pow loss (a measure of cortical activation) in the ipsilesional hemisphere during the more affected wrist task compared to the same measures in the non-dominant wrist in controls. Less ipsilesional Pow loss in patients was correlated with smaller maximum wrist extension forces. There were no group differences in resting Pow or task-related changes in IHCoh. In both groups, resting Pow was mildly lateralized to the contralesional or dominant hemisphere. The reduction of resting IHCoh in patients reflects a loss of inter-hemispheric connectivity that may contribute to dystonia severity and decreased hand function.

In addition, decreased activation of the ipsilesional hemisphere in patients may contribute to wrist extension weakness. These results show measurable differences in EEG parameters that are related to functional outcomes and may be due in part to injury-related structural changes and subsequent maladaptive neuroplasticity. Future work will focus on how these neurophysiological measures are influenced by time after injury and therapeutic interventions. In addition, it will be important to determine whether these EEG measures are specific to dystonia, or are common to childhood stroke or cerebral palsy.

Disclosures: S.N. Kukke: None. **A. de Campos:** None. **D. Damiano:** None. **K. Alter:** None. **M. Hallett:** None.

Poster

718. Stroke: Imaging and Diagnostics II

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Program#/Poster#: 718.10/EE3

Topic: C.21.Stroke Recovery

Title: Multimodal assesment of the motor system for hand motor recovery prognosis in chronic stroke patients - Possibility of cortical mapping of hand synergies using navigated TMS

Authors: *M. NAZAROVA[1,2], M. PIRADOV[2], E. BLAGOVECHTCHENSKI[3], V. NIKULIN[3,4];
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Abstract: Hand motor recovery prognosis in chronic stroke patients is crucial to determine the extent of rehabilitation potential and therefore to develop a realistic individual rehabilitation plan. One of the most important problem for hand motor recovery are pathological synergies including proximal muscle`s involvement in the precise hand movements. Thereby non-invasive mapping of proximal and distal upper limb muscle`s cortical representation which became available owing to accesibility of MRI-navigated transcranial magnetic stimulation (nTMS) may be a possible new biomarker for hand motor recovery in stroke patients. The aim of the study was to perform a multimodal assessment and compare a predictive role of different structural and functional biomarkers including cortical representation of upper limb muscles and their overlap for hand motor recovery in chronic stroke patients. Total of 18 patients with the only chronic

(more then 6 months) supratentorial ischemic stroke and various severity of hand paresis were enrolled (10 females, medium age 44,0±8,0) (the study is in progress). The assessment included investigation of structural parameters such as corticospinal tract (CST) integrity measured by diffusion tensor imaging (DTI) and TMS and functional assessment including analysis of BOLD response of the cortical areas induced by passive raising of the index finger of the paretic hand, cortical excitability level assessment in both hemispheres reflected by short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) phenomena and nTMS motor mapping of one intrinsic hand muscle - m. Abductor Pollicis Brevis (APB) and one extrinsic hand muscle - m. Extensor Digitorum Communis (EDC). A control group of 14 healthy volunteers (7 females, mean age 36,6±15,2) completed functional MRI passive motor task, nTMS mapping of EDC and APB and an investigation of SICI and ICF phenomena. Hand motor function was analysed by Fugl-Meyer assessment scale and Action Research Arm Test (ARAT). There was a strong correlation between hand motor function and fractional anisotropy asymmetry in the internal capsule. Motor thresholds and cortical maps of the APB and EDC muscles representations were different in patients with different hand paresis severity and not always overlapped with fMRI activation maps despite the data in the control group. In the controls motor representations of EDC were wider and zones of APB more local in contrast to patient group.

Disclosures: **M. Nazarova:** A. Employment/Salary (full or part-time):; Higher School of Economics, Department of Psychology, Centre for Cognition & Decision Making. **M. Piradov:** None. **E. Blagovechtchenski:** A. Employment/Salary (full or part-time):; HSE, Moscow. **V. Nikulin:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 719.01/EE4

Topic: D.01. Chemical Senses

Support: NIH DC010915

NIH DC005676

Title: Differential serotonin action on two classes of glomerular inhibitory interneurons

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Abstract: The first site for synaptic integration of olfactory sensory signals occurs within olfactory bulb glomerular circuits. Glomerular circuits mediate the input-output transformation of sensory information to higher brain regions and are themselves targeted by feedback projections from brain modulatory systems. We previously demonstrated that serotonin (5HT) directly and strongly excites external tufted cells (ETCs), key excitatory neurons in the glomerular circuit. Here, we investigated the action of 5HT on the two major inhibitory neuron classes of the glomerular microcircuit, GABAergic periglomerular cells (PGCs) and the GABAergic/dopaminergic short axon cells (SACs). PGC and ETC projections are restricted to a single glomerulus, while SACs project laterally onto 10s to 100s of glomeruli. Using mice that express GFP under the control of the glutamic acid decarboxylase-65kDa promoter (GAD65gfp) we measured whole cell responses to 5HT of GAD65-positive PGCs. None of the recorded PGCs (n=17) responded directly to 5HT. Serotonin evoked elevated EPSC frequencies in GAD65+PGCs but addition of glutamatergic blockers abolished 5HT-mediated EPSC increases, consistent with our previous finding that 5HT excites ETCs, which in turn, provide glutamatergic input to PGCs. In contrast, robust direct 5HT depolarizing currents were observed in SACs (n=8), identified with a line of mice expressing GFP under the control of the tyrosine hydroxylase promoter. The 5HT-mediated depolarization was associated with a drop in input resistance, suggesting a net opening of ion channels in the SAC membrane. The selective action of 5HT on SACs was abolished by 5HT2 receptor antagonists and mimicked by 5HT2 agonists. Thus, within the glomerular microcircuit 5HT directly excites both ETCs and SACs and indirectly increases excitatory drive on PGCs through its actions on ETCs. As ETCs also drive SACs, a major action of 5HT may be to preferentially enhance the impact of interglomerular circuitry.

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Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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UMBC/UMB Meyerhoff Graduate Fellow

Title: Understanding the main olfactory bulb circuitry using intrinsic flavoprotein fluorescence imaging

Authors: *C. UYTINGCO, A. C. PUCHE, S. D. MUNGER;
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Abstract: The olfactory system can be divided into distinct subsystems based on the chemosensory receptors they express, the stimuli to which they respond to, and the connections they make in the brain. However, it is unclear whether specialized subsystems within the main olfactory system utilize similar or unique strategies to process olfactory information in the main olfactory bulb (MOB). By imaging changes in the endogenous fluorescence of mitochondrial flavoproteins that accompany neuronal-associated increases in metabolic load, we are able to map the functional circuitry associated with individual glomeruli in MOB slices without the use of external dyes or genetically encoded indicators. Using horizontal MOB slices from 3-6 w.o. mice, electrical stimulation (0.5-6s, 10-50Hz, 10-100uA) of individual canonical glomeruli elicited robust flavoprotein signals in both the glomerular layer (GL) and external plexiform layer (EPL). Flavoprotein signal profiles from the GL and EPL shared the same biphasic stimulus-dependent response, but differed in signal amplitude, duration, and kinetics. Recordings obtained under either high (10x) or low (4x) magnification showed a bilateral signal spread from the stimulated glomerular circuit in both the GL (150µm) and EPL (300µm). Bath application of 10M gabazine increased the signal amplitude and lateral spread 2-fold in both the GL and EPL, indicating a strong influence of GABAergic interneurons on limiting the response and lateral spread. Surgical microcuts of the GL and/or EPL differentially impacted the unilateral spread of flavoprotein fluorescence, suggesting that both the interglomerular-interneuron and mitral-granule-mitral pathways contributed to lateral communication in MOB slices. Comparative studies between canonical and necklace glomeruli, the latter targeted by GC-D-expressing olfactory sensory neurons, are ongoing. Preliminary studies show signal amplitude and spread following stimulation of single necklace glomeruli in the presence of gabazine are similar to those seen with the canonical glomeruli stimulation. This study highlights the ability of intrinsic flavoprotein fluorescence imaging to understand the functional connectivity of neuronal circuits and for revealing basic information processing strategies within the olfactory bulb.

Disclosures: **C. Uytingco:** None. **A.C. Puche:** None. **S.D. Munger:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

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Program#/Poster#: 719.03/EE6

Topic: D.01. Chemical Senses

Support: DMS-1200004

R01-DC02751

Title: Both intrinsic and circuit mechanisms regulate the afterhyperpolarization phase in the projection neurons of moth antennal lobe

Authors: *H. LEI[1], Y. YU[2], J. G. HILDEBRAND[3], A. Y. RANGAN[4];
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Abstract: Afterhyperpolarization (AHP) following action potentials is critical for neuronal repolarization, frequency adaptation and gain control. In the antennal lobe of the male tobacco hawkmoth Manduca sexta, the pheromone responsive projection neurons (PNs) exhibit AHP as part of their responses to the olfactory stimulation with the natural conspecific female sex pheromone. To investigate the mechanisms underlying the AHP of these neurons, we conducted a series of electrophysiological recordings on PNs in conjunction with pharmacological applications using potassium channel blockers, GABA-A receptor antagonists and GABA transporter blockers. The results demonstrate that AHP may be regulated by both intrinsic and extrinsic mechanisms. The quaternary salts of bicuculline, potent blockers of SK channels, completely block AHP, resulting in long lasting firing upon odor stimulation. Picrotoxin (GABA-A receptor antagonist) and L-DABA (GABA transporter inhibitor) surprisingly increased the AHP duration, indicating a contribution of GABA mechanisms. These observations cannot be explained by direct actions of picrotoxin on GABA-A receptors on PNs, which would cause decreased inhibitory currents on these PNs. A plausible explanation is that disinhibitory circuits regulate the excitability of PNs, involving two GAGAergic interneurons; picrotoxin may increase the activity level of the 1st interneuron, which results in less inhibition of the 2nd interneuron and consequently, more inhibition on the PNs. These results suggest that PNs' excitability is constantly under the regulation of GABA mechanisms. To analyze the circuit events quantitatively, we constructed a dynamical model containing olfactory sensory neurons, PNs and GABAergic interneurons. The modeling study reveals how the circuits produced a well-

balanced excitation and inhibition on PNs so that these neurons can dynamically adjust excitability. Supported by DMS-1200004 to HL and AR; R01-DC02751 to JGH.

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Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 719.04/EE7

Topic: D.01. Chemical Senses

Support: NIH DC006441

NIH DC011423

Title: High-frequency sniffing reformats odor representations by altering the balance of excitation and inhibition in olfactory bulb output neurons

Authors: ***M. WACHOWIAK**[1], M. DIAZ-QUESADA[1], M. N. ECONOMO[1], K. R. HANSEN[2];
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Abstract: Olfactory information is encoded by patterns of action potential firing which are temporally structured around the respiratory cycle. Mitral and tufted (MT) cells of the olfactory bulb (OB), which receive primary sensory input and project to olfactory cortex, show strong respiratory patterning that is driven by inhalation and shaped by the relative balance and timing of excitatory and inhibitory synaptic inputs. Repeated odorant sampling at higher frequencies - i.e., ‘sniffing’, a hallmark of active olfactory sensing - may affects this balance and thus shape sub- and suprathreshold MT cell responses to odors. Here, we explored this possibility by obtaining whole-cell current clamp recordings from MT cells while varying the frequency of odorant inhalation in the anesthetized mouse using an 'artificial sniff' paradigm that allowed precise comparison of inhalation-linked response patterns across cells. We characterized effects of ‘sniff’ frequency on MT cell responses to over 85 cell-odor pairs, testing frequencies of 1, 3 and 5 Hz, which spans a range from resting respiration to active odor sampling. Inhalation-linked response patterns were characterized from ‘sniff-triggered’ average membrane potential changes as well as spike histograms. At 1 Hz inhalation, MT cells showed diverse responses consisting of distinct and reproducible combinations and sequences of depolarization and hyperpolarization

underlying inhalation-driven spiking patterns. Hyperpolarizing response components were equally likely for tufted as for mitral cells. Notably, increasing inhalation frequency substantially altered 'sniff-triggered' average responses in the majority of cell-odor pairs, with the relative strength of excitatory (depolarizing) versus inhibitory (hyperpolarizing) components often changing dramatically with frequency. However, the particular effect of frequency varied for different cells and even for different odorant responses in the same cell. As a result, the pattern of activity across a population of MT cells was different for the same odorant sampled repeatedly at different frequencies. In contrast, such diverse effects of frequency were not seen for MT responses evoked by repeated current pulses or optogenetic stimulation of all sensory inputs, suggesting that they arise from the pattern of odorant-evoked input to OB glomeruli relative to a particular MT cell rather than intrinsic differences among cells. These results suggest that sampling behavior alone can reformat early sensory representations, possibly to optimize sensory perception during repeated stimulus sampling.

Disclosures: M. Wachowiak: None. **M. Diaz-Quesada:** None. **K.R. Hansen:** None. **M.N. Economo:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Yale University James Hudson Brown - Alexander Brown Coxe Fellowship

Title: *In vivo* imaging of targeted cell populations in the mouse olfactory bulb

Authors: *D. A. STORACE[1], O. R. BRAUBACH[2,3], Y. CHOI[2], L. B. COHEN[1,2,3], U. SUNG[2]; [1]Cell. and Mol. Physiol., Yale Univ., New Haven, CT; [2]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [3]NeuroImaging Cluster, Marine Biol. Lab., Woods Hole, MA

Abstract: Genetically encoded voltage indicators can be used to optically monitor electrical activity from genetically distinct populations of neurons. The FP voltage sensor ArcLight has been used to study intact neural circuits in *Drosophila* and *C. elegans*, and most recently, the mouse olfactory bulb. The goal of the present study was to genetically target ArcLight to mitral/tufted cells in the mouse olfactory bulb, and measure their population response to odorant presentation. A floxed version of the ArcLight AAV1 vector was developed, and was injected into Pcdh21-Cre transgenic mice, which resulted in expression restricted to mitral/tufted cells. ArcLight signals were measured using widefield epifluorescence imaging in anesthetized mice in response to odorants. ArcLight had sufficient signal size and temporal resolution to resolve the spatio-temporal activity elicited by individual consecutive sniffs of an odorant in single trials. ArcLight can be genetically targeted to specific cell types using Cre/LoxP recombination and can report population activity elicited by individual sniffs of an odorant.

Disclosures: D.A. Storace: None. **O.R. Braubach:** None. **L.B. Cohen:** None. **U. Sung:** None. **Y. Choi:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

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NIH RO1 DC006640

Title: Cellular and population analyses of signal filtering at olfactory bulb glomeruli

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Abstract: GABAergic periglomerular (PG) cells in the olfactory bulb receive direct input from olfactory sensory neurons (OSNs) while also targeting glutamatergic external tufted (ET) cells that mediate feed-forward excitation of output mitral cells (MCs). It has been postulated that intraglomerular inhibition mediated by PG cells provides an alternate mechanism to lateral inhibition to decorrelate similar odors (Cleland & Linster, 2012). PG cells, which have a high input resistance (Puopolo & Belluzzi, 1998; Hayar et al., 2004), should be preferentially

activated when a glomerulus receives weak OSN input (e.g., due to an “off-target” odor), resulting in inhibition of the glomerulus. Stronger inputs, in contrast, should excite excitatory elements at the glomerulus sufficient to overcome inhibition. In this study, we used patch-clamp and imaging approaches in rat olfactory bulb slices to assess this hypothesis. We first tested the assumption that PG cells are more responsive than ET cells to current input. Following electrical stimulation of OSNs, PG cells indeed required much smaller monosynaptic excitatory post-synaptic currents (EPSCs; 141±26 pA, n = 8) to generate action potentials than ET cells (297±36 pA, n = 16; p = 0.0093). At the same time, however, the relatively large dendritic arbor of ET cells resulted in them having much larger EPSCs, which would favor ET cell excitation. During simultaneous pair-cell recordings (n = 4), the EPSCs in ET and PG cells, respectively, were 241 ± 134 pA and 45 ± 21 pA at a given stimulus intensity (4.5-10 μA). To test directly whether activation of PG cells or ET cells is favored at low levels of OSN activity, we used a population analysis of fura-2, AM associated calcium signals in VGAT-Venus transgenic rats, which selectively labels GABAergic cells. Weak OSN stimuli (5-20 μA) resulted in ~10 times more active presumed PG cells versus ET cells (ET to PG cell ratio = 0.09 ± 0.05, n = 8 glomeruli in 3 slices), although the relative number of active ET cells increased 3-fold (p = 0.0002) with stronger stimuli (10-50 μA) to a value (ET to PG cell ratio = 0.35 ± 0.05) that matched the total cell count ratio (ET to PG cell ratio = 0.37). Thus, the high input resistance of PG cells offsets their disadvantage of having a small number of OSN contacts, such that PG cells are mainly excited when a glomerulus receives weak input, but ET cell excitation catches up to PG cells with stronger input. These results support the hypothesis that the PG/ET cell microcircuit underlies a glomerular signal-filtering mechanism that could drive olfactory contrast enhancement.

Disclosures: **J.D. Zak:** None. **N.E. Schoppa:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Topic: D.01. Chemical Senses

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Title: High-speed recording of odor-evoked calcium transient in the olfactory bulb neurons using an AOD-based two-photon microscope

Authors: *R. HOMMA[1], X. LV[2,3], S. ZENG[2,3], S. NAGAYAMA[1];
[1]Univ. of Texas Med. Sch. at Houston, Houston, TX; [2]Dept. of Biomed. Engin., Huazhong Univ. of Sci. and Technol., Wuhan, China; [3]Britton Chance Ctr. for Biomed. Photonics, Wuhan Natl. Lab. for Optoelectronics, Wuhan, China

Abstract: A glomerulus of olfactory bulb is innervated by the axons of ~10,000 olfactory sensory neurons which express the same type of odorant receptors and the primary dendrites of tens of projection neurons (i.e. mitral and tufted cells). Furthermore, a glomerulus is innervated by hundreds of juxtaglomerular cells. For each glomerulus, all above neurons innervating the same glomerulus compose a functional unit (glomerular module). Although it is speculated that juxtaglomerular cells contribute to the early stage of odor information process within a glomerulus, their functional role is not well understood. To address the issue, it is essential to study a fair number of juxtaglomerular cells in the same module. However, identifying the neurons in the same glomerular module is technically challenging and, to date, only a small fraction of those neurons can be labeled. In this study, we attempted to identify a subset of juxtaglomerular cells that were associated with the same glomerulus, based solely on functional imaging. We used transgenic mice which express a calcium sensor protein GCaMP3 under the control of GAD2 (GAD65) promoter (the progenies of a GAD2-cre mouse crossed with a Flox-GCaMP3 mouse). In the glomerular layer, GCaMP3 expressing neurons are a subset of juxtaglomerular cells, but neither olfactory sensory neurons nor projection neurons. First, we used wide-filed imaging to record the spatial pattern of odor-evoked calcium signal in the dorsal part of olfactory bulb and selected 3 - 4 adjacent glomeruli that responded to different subset of odorants. Then, the odor-evoked response was recorded from approximately 20 GCaMP3 expressing neurons around the selected glomeruli, using two-photon microscope equipped with a 2D acousto-optic deflector (AOD). The system allowed us to record merely from a set of deliberately chosen locations in the field of view with a high sampling rate (>100 Hz). We were able to identify a group or distinct groups of neurons based on their highly correlated odor-response profiles, such as odor selectivity or the time course of calcium transient. Most, if not all, neurons in such a group surrounded the same glomerulus. The time course of odor-evoked calcium transient was homogeneous among the neurons within the same group, but often distinct among those in the different groups. The result suggests that the GABAergic juxtaglomerular cells associated with the same glomerulus show the similar time course of firing rate in response to an odor stimulus. It implies that glomerular module is a temporally organized neuronal network for the odor information process.

Disclosures: **R. Homma:** None. **S. Nagayama:** None. **S. Zeng:** None. **X. Lv:** None.

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719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Title: Molecular and functional characterization of lgr5 expressing cells in the olfactory bulb

Authors: A. H. MOBERLY, Y. YU, *M. MA;
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Abstract: LGR5 is a G-protein coupled receptor characterized by leucine-rich repeats in the extracellular domain. It has recently been identified as a *bona fide* marker for adult stem cells in multiple organs including the small intestine, colon, stomach, tongue, and cochlea of the inner ear. *Lgr5* is also expressed in the brain, but its role in the nervous system remains elusive. Using a gene-targeted *lgr5* reporter mouse line (Lgr5-EGFP-IRES-creERT2), we find that *lgr5* is highly expressed in the olfactory bulb (OB), especially in the glomerular layer. Double staining with other stem cell and neuronal markers reveals that *lgr5*+ cells in the OB are fully differentiated neurons instead of stem cells. Interestingly, *lgr5* expression does not completely coincide with any existing marker for specific subtypes of OB cells. To functionally characterize these cells we used targeted whole cell patch clamp recordings in OB slices. The *lgr5*+ cells in the glomerular layer fire action potentials in response to depolarizing current injection and display spontaneous miniature excitatory postsynaptic potentials (mEPSCs), suggesting that these neurons are integrated in OB neural circuits. We are currently investigating the functional significance of LGR5 and *lgr5* expressing neurons in the early stages of olfactory processing.

Disclosures: A.H. Moberly: None. **M. Ma:** None. **Y. Yu:** None.

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719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Title: Detection of components in an odor mixture is tuned by experience and changes in the antennal lobe

Authors: *E. MARACHLIAN[1,2], F. LOCATELLI[1];
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Abstract: Odors in nature are complex mixture in which irrelevant components may hide the presence of odors with predictive value. Moreover the meaning and relevance of each odor is not always fixed but may change depending on the animal's experience. Therefore, mechanisms must exist that allow animals optimize detection of the relevant odors according to its own experience. In agreement with this view we present behavioral experiments performed in honey bees showing that appetitive learning of an odor is reduced when during learning the odor is presented in a mixture with another odor that has been learned before. The reduction in learning is interpreted as an overshadowing-like effect, in which the presence of the learned odor hinders the perception of the novel one. This result poses the question about where this plasticity does occur along the olfactory processing pathway. Recent studies have revealed that odor representation in the antennal lobe, the first olfactory processing center in the insect brain, changes after olfactory experience. However the specific role of these changes remains still elusive. In the present work we test the hypothesis that plasticity in odor coding in the antennal lobes is related with increasing the gain and detection of relevant odors on top of background and informative odors. To test this hypothesis we performed calcium imaging in projection neurons of the antennal lobe to determine the neural activity patterns that represent the pure odors and the respective binary mixture in naïve and in trained honey bees. Using the patterns measured in naïve bees we established algorithms that allow accurate prediction of the pattern for the mixture based on the patterns measured for the pure components. The prediction algorithms obtained from naïve honey bees were applied to honey bees that had been trained on appetitive conditioning using as conditioned odor one of the components of the mixture. We found that the representation of the mixture in trained animals deviates from the predicted mixture. The deviation from the predicted mixture is in favor of the representation of the learned component and away from the representation of the novel component. The change in the representation of the mixture is evidenced by a reduction in activity of elements that encode the novel odor and not by an increase in activity of elements that encode the learned component. This might indicate that changes induced by training are caused by strengthening of the inhibitory interaction from the learned odor toward the novel odor. Similar solutions might be applicable in other sensory modalities or even in the design of biomimetic sensors.

Disclosures: **E. Marachlian:** None. **F. Locatelli:** None.

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719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Support: NIH Grant DC003195

Title: Differential modulation of mouse main olfactory bulb external tufted and mitral cell excitability by group I and II mGluRs

Authors: ***H. DONG**, M. ENNIS;
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Abstract: External tufted (ET) and mitral cells of the main olfactory bulb express several metabotropic glutamate receptor (mGluR) subtypes. Previous studies show that Group I mGluR agonist such as DHPG robustly increases the strength and frequency of ET cell bursting and rhythmic membrane potential/current oscillations. ET cells play a key role in synchronizing intraglomerular activity. Here, we examined how Group I and II mGluRs coordinately regulate ET and mitral cell activity. We found that the effect of DHPG on ET cells was accompanied by an increase in the amplitude and frequency of long-lasting depolarizations (LLDs) and spike bursts in mitral cells. As expected from these findings, paired recordings showed that DHPG increases synchronous activity of mitral cells associated with the same glomerulus. As previously reported, a non-selective group II mGluR agonist (L-CCG-I) also increased the strength and frequency of ET cell bursting, Surprisingly however, both L-CCG-I and a selective group II mGluR agonist DCG-IV dampened LLDs and spike bursts in mitral cells. These effects also occurred when L-CCG-I was applied in the presence of APV and gabazine. Thus, the suppression of mitral cell spike bursts and LLDs by L-CCG-I does not appear to be due to changes in the degree of NMDA or GABAa receptor activation in the network. LLDs are triggered by regenerative glutamate release from the apical dendrites of ET and mitral cells. Together, these results suggest that L-CCG-I may reduce glutamate release from ET and/or mitral cells. Consistent with this, L-CCG-I reduced autoexcitatory currents evoked by intracellular depolarization of mitral cells. These results indicate that group I and group II mGluRs differentially modulate ET and mitral cell excitability, and that Group II mGluRs have multiple effects on excitatory neurons in the glomerulus. Supported by PHS grant DC003195.

Disclosures: **H. Dong:** None. **M. Ennis:** None.

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Title: Alterations in the intrinsic and synaptic properties of olfactory bulb principal cells in Cntnap2 knockout mice

Authors: ***M. A. GERAMITA**[1], N. N. URBAN[2];
[1]Biol. Sci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: A prominent feature of autism spectrum disorders (ASDs) is dysfunction in sensory processing. Recent evidence suggests that these changes may ultimately arise from unreliable cortical responses to sensory stimuli (Dinstein 2012). It is unknown how specific genes known to increase risk for ASDs alter neuronal physiology and ultimately create a pathologically unreliable neural circuit. Ongoing work from our lab (Wen 2014) has found that in a mouse model of autism, (the contactin-associated protein-like 2 - CNTNAP2 knockout) reliability of odor-evoked responses is decreased. Here we explore electrophysiological changes to olfactory bulb principal cells (mitral and tufted) in CNTNAP2 knockout mice that may contribute to unreliable odor-evoked responses *in vivo*. Using whole-cell recordings from olfactory bulb slices, we assessed differences in the synaptic and intrinsic properties of mitral and tufted cells between CNTNAP2 (+/-) heterozygote (HET) and C57BL/6 (WT) animals. To assess synaptic properties of sensory neuron inputs to tufted cells, we measured paired pulse ratio (PPR) across 4 interstimulus intervals (50, 100, 500, 1000 ms) as an indicator of neurotransmitter release probability. Compared to WTs, CNTNAP2 HETs showed significantly less paired pulse depression (HETs have a 25% higher PPR across 4 interstimulus intervals) indicating a lower probability of transmitter release from primary olfactory synapses. To assess intrinsic properties, we measured the excitability and action potential properties of mitral cells. Compared to WTs, CNTNAP2 HETs showed a variety of significant differences that included: 1) 80% increase in the gain of the FI curve, 2) 35% increase in peak instantaneous firing rate, 3) 45% lower rheobase, 4) 7mV decrease in action potential threshold, 5) 33% decrease in

afterhyperpolarization amplitude. Our findings indicate that CNTNAP2 plays an important role in both the intrinsic and synaptic physiology of the olfactory bulb. These findings begin to dissect the circuit-level alterations that contribute to the autistic endophenotype of unreliable sensory-evoked responses.

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719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Support: DC010915

DC005676

Title: Cholinergic modulation of glomerular circuits sculpts olfactory bulb output

Authors: S. LIU[1], Z. SHAO[1], M. ROTHERMEL[2], M. WACHOWIAK[2], *A. C. PUCHE[1], M. T. SHIPLEY[1];
[1]Dept. of Anat. & Neurobio., Univ. of Maryland, Baltimore, MD; [2]Dept. of Neurobio. and Anat., Univ. of Utah, Salt Lake City, UT

Abstract: Cholinergic inputs from the basal forebrain heavily target olfactory bulb glomeruli, the initial site of synaptic integration in the olfactory system. Both nicotinic (nAChRs) and muscarinic (mAChRs) acetylcholine receptors are highly expressed in glomeruli. Activation of nAChRs directly excites both mitral/tufted cells (MTCs) and external tufted cells (ETCs), the two major excitatory neuron types that drive glomerular circuits. However, the functional roles of mAChRs in glomerular circuits are unknown. We show that restricted glomerular application of ACh causes, rapid, brief nAChR-mediated excitation of both MTCs and ETCs. This excitation is followed by mAChRs-mediated inhibition, which is blocked by GABAA receptor antagonists suggesting engagement of periglomerular cells (PGCs) and short axon cells (SACs), the two major glomerular inhibitory neurons. Indeed, selective activation of glomerular mAChRs, with iGluRs and nAChRs blocked, enhances sIPSCs in MTCs and ETCs. This suggests that mAChRs recruit glomerular inhibitory circuits. Surprisingly, selective activation of mAChR hyperpolarizes the somas of both PGCs and SACs, but increases mIPSCs in all glomerular

neurons suggesting that mAChRs enhance GABA release from PGC and/or SAC dendrites. Together, our results indicate that, cholinergic modulation of glomerular circuits causes initial brief excitation of MC/ETCs, mediated by nAChRs followed by an epoch of inhibition mediated directly by mAChRs on PGCs/SACs and indirectly by MC/ETC excitation of PGCs/SACs. Such circuit-level modulation could phasically enhance the sensitivity of glomerular outputs to odorants when ACh is released from centrifugal afferents, an effect consistent with our *in vivo* findings. Supported by NIH DC010915, DC005676

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719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Title: A tale of two transmitters: Effects of dopamine and GABA on the olfactory bulb microcircuit

Authors: ***C. VAAGA**, J. T. YORGASON, J. T. WILLIAMS, G. L. WESTBROOK; Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Co-transmission, the ability of a neuron to release multiple neurotransmitters, has been recognized in many circuits including the retina, ventral tegmental area and olfactory bulb. The functional role of co-transmission within intact circuits, however, has been difficult to fully resolve. In the olfactory bulb, *in vitro* isolation of tyrosine hydroxylase positive (TH+) periglomerular interneurons revealed that these cells release dopamine and GABA, albeit over very different timescales (Borisovska et al., 2013); suggesting that these neurotransmitters are packaged into non-overlapping pools of synaptic vesicles. The functional importance of

dopamine/GABA co-transmission within the olfactory bulb microcircuit, however, is not well understood. To activate the ensemble of dopamine/GABA neurons surrounding each glomerulus, we expressed channelrhodopsin under control of the DAT promoter. We then used focal electrical stimulation of single glomeruli in combination with optogenetic stimulation to explore the role of dopamine/GABA co-transmission on monosynaptic excitatory postsynaptic currents (EPSCs) in mitral cells. Here we show that endogenously released dopamine and GABA attenuated the axodendritic, monosynaptic EPSC. Although the mitral cell EPSC has a prominent slow component resulting from dendrodendritic excitation, optogenetically released dopamine and GABA preferentially attenuated the fast component. Preliminary studies using fast-scanning cyclic voltammetry to directly measure endogenous dopamine release revealed that a dopamine signal was detected for seconds after brief optogenetic stimluation. We suggest that although both dopamine and GABA attenuate the evoked EPSC, they act on different timescales and therefore differentially alter circuit processing.

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719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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CONACyT: 289638 to V V-B

Title: Novel connectivity within the accessory olfactory bulb and between the main and accessory olfactory bulbs

Authors: V. VARGAS-BARROSO, F. PEÑA-ORTEGA, B. ORDÁZ, *J. LARRIVA-SAHD; Dept. of Developmental Neurobio., Inst. de Neurobiologia UNAM, Queretaro, Mexico

Abstract: In most mammals detection of volatile and pheromonal stimuli relies on two major olfactory pathways: the main- (MOS) and accessory-olfactory systems (AOS), respectively. Originally thought to be separated modalities, cumulating evidence supports a combined possibly synergic interaction between them. While a functional overlap between the MOS and AOS has

been extensively documented, structural and physiological substrates for such interaction have recently been emerging. In spite of the central anatomical convergence of both MOS and AOS, no direct interface between them has been detected. We searched for electrophysiological evidence for a direct interaction between the main (MOB) and accessory olfactory bulbs (AOB) and, then, visualized recorded neurons as they had previously been filled with biocytin. All recordings were performed in olfactory bulb slices from adult albino rats and included neurons from the anterior half of the accessory olfactory bulb (aAOB) and dorsal main olfactory bulb (dMOB). RESULTS: Single cell recordings of principal cells (PC) in aAOB following dMOB stimulation revealed antidromical activation of them. Additionally aAOB-PCs were orthodromically activated following stimulation of the posterior AOB (pAOB). In fact, stimuli placed in the pAOB elicit monosynaptic excitatory postsynaptic potentials in aAOB neurons. Posthoc visualization of biocytin-filled neurons revealed that AOB recorded neurons correspond to principal cells. It is concluded that: 1. The monosynaptic link documented here provides direct evidence for an anatomical interaction between the AOB and dMOB 2. The aAOB and pAOB are linked by PC axon collaterals from the latter.

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Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Support: NIH Grant R01 DC013329

Title: Imaging odor representation by olfactory bulb granule cells at subcellular resolution

Authors: *M. WIENISCH, V. N. MURTHY;
Mol. & Cell. Biol, Harvard Univ., CAMBRIDGE, MA

Abstract: The first sensory stages of the brain are thought to format relevant environmental information in an efficient manner that facilitates easy and flexible extraction by downstream areas. The olfactory bulb (OB) is the first circuit processing station for odor information, where inputs from olfactory sensory axons are processed by a complex network of neurons before mitral/tufted (M/T) cells carry the information to higher brain areas. Local processing in the

deeper layers of the OB involves axon-less interneurons called granule cells (GCs), which are at least 10 times more numerous than M/T cells. The exact function of GCs in odor processing remains unclear, but they are thought to play a role in temporal patterning of activity in M/T cells and context dependent lateral interactions within the OB. To address key questions about the spatiotemporal dynamics of GC activity and its relation to sensory stimuli we imaged odor responses in somata as well as dendrites of a large population of GCs using multiphoton microscopy and calcium imaging in mice. We found that odor responses in GCs were temporally diverse, and the overall density of activation of GCs was highly correlated with the extent of glomerular activation. Increasing concentrations of single odorants led to increasing overall population activity, but some individual GCs had non-monotonic relations due to local inhibitory interactions. Odor responses were readily observed in GC apical dendrites, the sites of excitatory synaptic inputs from principal neurons, and dendritic responses were more broadly tuned than somatic ones. Individual dendritic segments could respond independently, revealing their capacity for local processing. Collectively, the response properties of GCs point to their role in specific and local inhibition, in contrast to global normalization or gain control proposed for other interneurons in the OB.

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Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Title: Circuit mediating inhibition of olfactory bulb periglomerular cells

Authors: A. SANZ-DIEZ, *D. DESAINTJAN;
INCI, CNRS UPR 3212, Strasbourg, France

Abstract: The olfactory bulb (OB) is the first relay station in the brain for odor processing. It receives sensory afferents from olfactory sensory neurons (OSN). This information is transmitted to mitral and tufted cells, the principal output neurons of the bulb, within anatomical structures called glomeruli. Each glomerulus is surrounded by periglomerular (PG) cells that mediate intraglomerular inhibition. We recently characterized a specific PG cell subtype that plays a

dominant role in mediating OSN-evoked intraglomerular inhibition of mitral and tufted cells (Najac et al. submitted). This PG cell subtype that expresses EYFP in KV3.1-EYFP transgenic mice represents 20-30% of all PG cells, is axonless and projects its dendrites into a single glomerulus. EYFP(+) PG cells, like most PG cells, receive spontaneous inhibitory synaptic inputs (IPSCs) at an average frequency of 1.17 ± 1.05 Hz (n=9). We have examined the circuit mediating this inhibition. Using whole-cell patch-clamp recordings in horizontal slices of olfactory bulb from KV3.1-EYFP transgenic mice, we found that stimulation of OSN that produced excitatory postsynaptic currents (EPSC) in EYFP(+) PG cells voltage-clamped at negative holding potentials (Vh = -75 mV) did not evoke any IPSCs when the cells were clamped around the reversing potential for excitation (Vh=0 mV, n=10). In contrast, stimulation within distant (>200 µm) glomeruli produced a gabazine-sensitive monosynaptic IPSC (average amplitude 58 ± 36 pA, n=10) resistant to NBQX and D-AP5. Evoked IPSCs had 20-80% rise-time (0.49 ± 0.12 ms) and half-width (14.9 ± 4 ms) that were similar to those of spontaneous IPSCs (0.56 ± 0.08 ms and 15.5 ± 2.63 ms, respectively). These results suggest that inhibitory inputs onto EYFP(+) PG cells are not generated by the glomerular network they are associated with but instead are provided by neurons with axons running within the glomerular layer. Thus, we suspect short-axon cells located in infra mitral cell layers (deep SA cells) or in the glomerular layer (superficial SA cells) that have an axon that ramifies across several glomeruli to be the neurons inhibiting EYFP(+) PG cells.

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Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Support: PhD fellowship by the French ministry of research

Title: Olfactory-feeding crosstalk: Probing plasticity in the olfactory bulb of obese mice by Manganese-Enhanced MRI

Authors: *Y. CHELMINSKI[1], C. MARTIN[1], C. SEZILLE[1], A. GENOUX[1], C. SEBRIÉ[2], S. SCOTTO-LOMASSESE[3], H. GURDEN[1];
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Abstract: The olfactory system is crucial for feeding behavior: it allows the processing of olfactory cues related to food location and palatability. Interestingly, receptors to anorexigen (leptin and insulin) and orexigen (ghrelin) hormones found in the hypothalamus are also expressed in the main olfactory bulb (MOB), the first stage of olfactory processing in the brain, suggesting that feeding state has an impact on odor representation. Indeed, these hormones were reported to regulate the activity of MOB cells *in vitro*. However, it is unknown whether obesity could impact MOB function *in vivo*. Here we tested the effects of obesity on MOB activity. Ob/ob mice are deficient in leptin from birth and are widely used as a murine model for obesity since they are hyperphagic and rapidly obese. We used a functional neuroimaging technique, Manganese Enhanced MRI, to monitor food odor-evoked activity in the MOB of these mice. MEMRI uses manganese as a contrast agent. This ion is an analog of calcium and penetrates into the olfactory neurons that are activated by odorants. Then manganese is transported and accumulates in specific regions of the MOB making possible MEMRI recordings of spatial maps in the MOB. We found that the number of pixels activated in the MOB in control conditions with no odor stimulation is higher in ob/ob mice compared to wild type (each group n=5). We also observed that the number of food odor-activated pixels is higher in the ob/ob mice (each group n=5). Moreover, injection of leptin strongly reduces the number of food odor-evoked pixels (each group, n=4). To pinpoint what cellular/molecular mechanisms can be responsible for these activity changes in the MOB, we first quantified mRNA expression of neuronal (OMP), astrocytic (GFAP) and microglial (IBA1) molecular targets by RT-PCR, but did not find any significant changes between ob/ob (n=10) and control mice (n=10). We also quantified bulbar adult neurogenesis and found that 21 days after BrdU injections, a cell birth marker, ob/ob mice (n=6) showed an increased number of both new periglomerular and granular cells as compared to control (n=10), suggesting that leptin regulates new neuron elimination. Finally, ob/ob mice displayed an evident lack of motivation for the learning of an odor discrimination task. Local network activity within the MOB in ob/ob mice is currently under analysis.

Disclosures: **Y. Chelminski:** None. **C. Martin:** None. **C. Sezille:** None. **A. Genoux:** None. **C. Sebrié:** None. **S. Scotto-Lomassese:** None. **H. Gurden:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: D.01. Chemical Senses

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Title: Birthdate-dependent segregation of mitral cell dendrites in mouse olfactory bulb

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Abstract: Mitral cells are the projection neurons of the olfactory bulb (OB). Their cell bodies are located in the mitral cell layer (MCL), and axons project broadly to the olfactory cortex. Mitral cells possesses two types of dendrites: 1) a primary dendrite that extends radially and arborizes in a single glomerulus where it receives primary input from olfactory sensory neuron axons; and 2) secondary dendrites that extend horizontally in the external plexiform layer (EPL) where they make reciprocal dendrodendritic synapses with granule cells. Mitral cells are often presented as an OB counterpart of cortical pyramidal neurons, whose morphological and molecular properties are partly determined by their timing of genesis in the developing brain. We previously reported that early- and late-born mitral cell somata localized differently in the dorsomedial and ventrolateral MCL, and that the olfactory tubercle receives heavier axonal input from late-born mitral cells (Imamura et al. *Nat. Neurosci.* 14, 331, 2011). Here, we further hypothesized that mitral cell dendritic organization is also regulated by generation timing. To test this hypothesis, we introduced the plasmid having *gfp* cDNA (pGFP) into the mouse OB using *in utero* electroporation. By changing the timing of electroporation after fertilization, mitral cells in the accessory olfactory bulb (AOB) and the main olfactory bulb (MOB), and tufted cells, another OB projection neuron, were labeled with different proportions. The electroporation performed at embryonic day (E) 10 preferentially labeled AOB mitral cells and MOB mitral cells in dorsomedial MCL. While the E12 electroporation introduced pGFP preferentially into OB mitral cells in ventrolateral MCL and tufted cells. Combining these data with BrdU injections, we further confirmed that E10 and E12 electroporation preferentially labeled early- and late-born projection neurons, respectively. These results demonstrate that *in utero* electroporation can introduce pGFP into distinct subsets of OB projection neurons based on their birthdates. Then, comparing the GFP-labeled processes in the EPL, we found that early- and late-born projection neurons extend their secondary dendrites in the deep and superficial EPL, respectively. Similar segregation has been suggested between mitral and tufted cells. However, by reconstructing the morphologies of GFP-labeled cells, we revealed that some late-born mitral cells that have cell bodies in the MCL extend secondary dendrites predominantly in the superficial EPL. Our observations indicate that timing of neurogenesis also regulates dendritic extension, and therefore connectivity, of mitral cells.

Disclosures: F. Imamura: None. **C.A. Greer:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 719.19/EE22

Topic: D.01. Chemical Senses

Title: Broad sensory activity rapidly alters the intrabulbar map

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Abstract: Axons from the olfactory sensory neurons (OSN) expressing the same olfactory receptors (OR) converge on a pair of glomeruli (iso-functional glomeruli) in each olfactory bulb (OB), creating a mirror-symmetric glomerular map. Neural connections between OSNs, mitral/tufted cells and interneurons both within and beneath each glomeruli form a columnar structure that serves as a basic functional unit for olfactory information processing. Previously we showed that iso-functional odor columns are specifically linked to one another through a set of intrabulbar projections (IBPs) mediated by tufted cells to form an intrabulbar map. Like many sensory circuits, the intrabulbar map develops postnatally through an activity-dependent refinement process, however, with IBPs the structural plasticity persists throughout adulthood. This feature has primarily been studied through naris closure experiments that easily block odor-induced activity but also affect survival of OSNs and OB interneurons. In the present study we sought to examine the role of activity in shaping IBPs through a more direct approach. Using UBI7 mutant mice in which the I7 receptor is expressed in all mature olfactory sensory neurons we could quickly activate the entire olfactory system for brief periods of time using the odorant Octanal (I7-ligand), and determine the effects on intrabulbar map organization. Notably, since the I7 receptor is expressed at low levels, it does not block the expression of endogenous ORs and does not alter the glomerular map. Our data show that following daily exposure to octanal, IBPs in the UBI7 mice exhibited significant axonal broadening within just one week while the glomerular organization remained intact. In addition, we found that IBPs in the octanal-exposed UBI7 mice could quickly re-refine to control levels of specificity upon removal of octanal. Importantly, we did not see indications of large neuronal loss that is typically associated with sensory deprivation. Thus, we conclude that activity plays an instructive role in the IBP refinement and that changes in activity dynamically alter their specificity.

Disclosures: **U. Park:** None. **M. Nguyen:** None. **D. Cummings:** None. **L. Belluscio:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Topic: D.01. Chemical Senses

Support: NIH Grant F31DC013490

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Title: Feedforward inhibition regulates granule cell recruitment in the mammalian main olfactory bulb

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Abstract: Sensory encoding in the main olfactory bulb (MOB) critically depends on granule cell (GC)-mediated lateral inhibition to: 1) decorrelate principal neuron activity downstream of overlapping afferent input, and 2) temporally pattern principal neuron activity within oscillatory network rhythms. Consequently, disruption of GC recruitment by perturbation of either synaptic excitation or inhibition onto GCs significantly alters MOB network rhythms and odor discrimination. Understanding how sensory input recruits GCs will thus be a crucial step in understanding olfaction, yet to date, no study has examined how GCs integrate feedforward synaptic excitation and inhibition to generate spiking output. Here, we have used patch clamp electrophysiology in acute MOB slices to investigate GC recruitment by integrated feedforward excitatory and inhibitory synaptic input. Stimulation of olfactory sensory neurons (OSNs) evoked robust and asynchronous excitatory postsynaptic currents (EPSCs) onto GCs, as previously observed. Subsequent depolarization of GCs to the EPSC reversal potential surprisingly revealed that the majority of GCs also received feedforward inhibitory postsynaptic currents (IPSCs), a previously unreported feature of the MOB circuit. Analysis of the average peristimulus time histogram confirmed that OSN stimulation significantly increased the incidence of IPSCs onto GCs ($p=0.004$, t-test; IPSC probability: 0.08 ± 0.03 vs. 0.25 ± 0.06, 100

ms before stimulation vs. 100 ms after stimulation [n=11], mean ± s.e.m.). IPSCs were most frequently detected at short latency (10-20 ms) following OSN stimulation, suggesting that a trisynaptic circuit mediates feedforward inhibition onto GCs. Supporting this hypothesis, deep short-axon cells, a population of MOB neurons that inhibit GCs, were rapidly recruited by OSN stimulation (first spike latency: 13.3 ± 4.3 ms [n=5], mean ± s.d.). To begin to investigate how feedforward inhibition regulates GC recruitment, we have confirmed in single and paired recordings that loading of GCs with 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS) via the recording electrode blocks synaptic inhibition in a cell autonomous manner, as measured by the ablation of spontaneous IPSCs (p=0.001, rank sum test; event rate: 1.5 ± 2.3 vs. 0.1 ± 0.1 Hz, control [n=24] vs. DNDS [n=7], mean ± s.d.) but not EPSCs (p=0.41; 3.8 ± 3.7 vs. 6.2 ± 6.4 Hz). Ongoing experiments are testing how intracellular block of synaptic inhibition influences the latency of GC spiking in response to static OSN stimulation and the synchronization of nearby GCs in response to dynamic OSN stimulation simulating physiological sniff-coupled input.

Disclosures: S.D. Burton: None. **N.N. Urban:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

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Topic: D.01. Chemical Senses

Support: DFG Grant KL 762/5-1

DFG Grant KL 762/6-1

Humboldt fellowship to BW

Title: Differences of calcium handling properties between different functional compartments of uniglomerular projection neurons

Authors: *D. FUSCA, A. PIPPOW, B. WARREN, H. WRATIL, P. KLOPPENBURG;
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Abstract: The antennal lobe of insects constitutes the first synaptic relay and processing center of olfactory information, received from olfactory sensory neurons located on the antennae. Complex synaptic connectivity between olfactory neurons of the antennal lobe ultimately determines the spatial and temporal tuning profile of (output) projection neurons to odors. Using

paired whole-cell patch-clamp recordings in the cockroach *Periplaneta americana* we characterized the excitatory synaptic interactions between cholinergic uniglomerular projection neurons (uPNs) and GABAergic type I local interneurons, both of which are key components of the insect olfactory system. Between uPNs and type I local interneurons we found short latency (< 2ms), rapid, strong excitatory cholinergic synaptic transmission that was coincident with single presynaptic action potentials. These results clearly show that uPNs have not only an important role in relaying processed olfactory information form the antennal lobe to higher brain centers, but also provide synaptic input to antennal lobe neurons during olfactory processing. Since highly localized cytosolic Ca^{2+} dynamics are involved in pre- and postsynaptic signal processing we started to investigate the Ca^{2+} handling properties in the glomerular neurites of uPNs. Using the added buffer approach combined with Ca^{2+} imaging and electrophysiological recordings we observed significantly different Ca^{2+} handling properties in the different functional compartments of uPNs. For example: Compared to the soma the Ca^{2+} extrusion rates were significantly larger in the glomerular neurites, while the Ca^{2+} binding ratios where similar in both compartments. Electrophyiological recordings combined with fast multiphoton Ca^{2+} imaging showed that the neuritic Ca^{2+} handling properties were sufficient that even fast electrophysiological activity was reflected in the voltage dependent cytosolic Ca^{2+} dynamics. Because Ca^{2+} regulates a multitude of cellular functions, and many aspects of information processing in single neurons are dependent on highly localized calcium domains, we consider the characterization of cellular parameters that determine cytosolic Ca^{2+} dynamics, as an important step towards a detailed understanding of the cellular basis of olfactory information processing at the single cell level. **This work was supported by an Alexander von Humboldt fellowship awarded to BW. Work in the Kloppenburg lab was supported by grants KL 762/5-1 and KL 762/6-1 from the deutsche Forschungsgemeinschaft.**

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Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

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Topic: D.01. Chemical Senses

Support: FPU-Spanish Ministry of Education Grant Ref: AP2009-3555

Title: Are there quantitative changes in the circuitry of the olfactory bulb in methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine -treated monkeys?

Authors: *T. LIBERIA VAYÁ[1], J. LANCIEGO[2], J. BLASCO-IBÁÑEZ[1], J. NÁCHER[1], E. VAREA[1], C. CRESPO[1];
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Abstract: It is well known that Parkinson's disease (PD) is a neurodegenerative disease characterized by the degeneration of dopaminergic nigrostriatal pathway in the brain. It could be for that reason that most of the published studies analyzing different aspects of Parkinson disease are focused on changes in the striatum. As a result of these researches, it is well established that complex neurochemical and morphological changes within the basal ganglia circuitry occur in the PD. Likewise, it has been widely demonstrated that olfactory dysfunction is a common and early symptom of PD and it could be used as an early biomarker for the diagnosis of this disorder. The neuropathologic changes responsible for olfactory dysfunction might involve different areas related to the olfaction at distinct levels. These could be: olfactory epithelium, olfactory bulb (OB), olfactory tract, primary olfactory cortices and their secondary cortices. All these areas have been studied under different points of views and methodology during the last years. It has been described that damage to other neurotransmitter systems, apart from the dopaminergic one, are likely related to olfactory dysfunction but these studies were carried out in other regions different from the OB. Indeed, the OB has been mainly analyzed regarding deposition of pathological proteins and there are few studies focused on the analysis of hypothetical morphological and quantitative changes that could occur in the circuitry of the OB. In order to determinate whether and how the OB is affected in PD we have used the OB of methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-treated monkeys as a model of parkinsonism. First, we have analyzed the density of subcortical inputs in the OB in control and MPTP conditions. Second, we have carried out a comparative quantitative study between both the glutamatergic and GABAergic circuits of the OB in both conditions. Third, quantitative studies of synaptophysin and gephyrin have been made in order to check if there were differences in the synaptic transmission of the OB in PD, The present results reveal that only the glutamatergic transmission in the external plexiform layer and the synaptic activity in the glomerular layer are modified in PD according to our quantitative studies.

Disclosures: T. Liberia Vayá: None. **J. Lanciego:** None. **J. Blasco-Ibáñez:** None. **J. Nácher:** None. **E. Varea:** None. **C. Crespo:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Topic: D.01. Chemical Senses

Support: NIH Grant R01 DC 008855

Title: Amplification of nicotinic receptor-mediated glomerular inhibition by signaling between periglomerular cells in the mouse olfactory bulb

Authors: *P. PARSA[1], R. D. D'SOUZA[2], S. VIJAYARAGHAVAN[3];
[1]Univ. of Colorado, Denver, Aurora, CO; [2]Anat. and Neurobio., Washington University, Sch. of Med., St. Louis, MO; [3]Univ. of Colorado, Sch. of Med., Aurora, CO

Abstract: The mouse olfactory bulb (OB) is characterized by high levels of nicotinic acetylcholine receptor (nAChR) expression, particularly in the glomerular layer. Previous work in our laboratory has shown that activation of glomerular nAChRs controls the propagation of olfactory nerve (ON) input to mitral cells (MCs), via an excitation-driven inhibitory feedback mechanism, so that relatively weak ON inputs are suppressed. A key process in this modulation is GABA release from periglomerular (PG) neurons on to MCs and external tufted (ET) cells. Here, we demonstrate that excitation of nAChRs results in a barrage of GABAergic postsynaptic currents (GPSCs) on PG cells presumably arising from surrounding PG cells. This effect is indirect, resulting from glutamate-driven excitation of PG cells via nAChR-driven activation of MCs and ET cells. Using optogenetic excitation, live slice imaging, and electrophysiological recordings, we show that GABA is depolarizing on PG neurons and has bidirectional effects on their firing activity. Using cell-attached recordings, we found that application of 25-50 μM GABA for 1-3 s induced a transient increase in action potentials in most PG cells. In some cells, 1-3 s application of GABA triggered a long lasting tonic increase in firing frequency. Under these conditions, a second application of GABA effectively abolished firing, suggesting that GABA modulation of PG cell firing is dynamic and state-dependent. Depolarizing a single PG cell led to a spread of calcium signals across multiple juxtaglomerular neurons due to GABA release and subsequent activation of $GABA_A$ receptors. Similarly, local GABA application at the edge of a glomerulus, triggered a barrage of GPSCs in distant PG cells. These results suggest that GABA release in the olfactory glomerulus can be amplified by a GABA-induced-GABA release (GIGR) mechanism. This amplification could lead to effective inhibition of all MCs within a glomerulus. We propose that during the attentional/anticipational phase of behavior, ACh release, via basal forebrain cholinergic activation, normalizes glomerular activity by efficient GIGR-induced inhibition of the local microcircuit. This inhibition sets the parameters of a high pass filter that efficiently attenuates weak odorant signals while allowing salient olfactory input to pass through for higher order processing.

Disclosures: **P. Parsa:** None. **R.D. D'Souza:** None. **S. Vijayaraghavan:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Topic: D.01. Chemical Senses

Support: NINDS Intramural Program 1ZIANS003002

Title: Activity-dependent plasticity in olfactory bulb dopaminergic neurons

Authors: ***B. GRIER**, C. CHEETHAM, L. BELLUSCIO;
NIH, Bethesda, MD

Abstract: The olfactory bulb (OB) displays remarkable plasticity that persists through adulthood, long beyond the plastic periods of other neural systems. Changes in olfactory sensory neuron (OSN) input to the OB have been shown to elicit profound effects on the morphology of both the OB and individual OB neurons. In particular, loss of OSN input results in a rapid downregulation of both tyrosine hydroxylase activity and dopamine production. It is unknown, however, how this loss of activity affects the structure and function of dopaminergic OB neurons, or through what mechanisms these changes may occur. In the present study we have analyzed the changes in dopaminergic OB neuron populations following downregulation of OSN input by way of reversible naris occlusion. Through a series of imaging experiments in transgenic animals we have recorded broad changes in OB dopaminergic neuron populations and are investigating the mechanisms underlying the observed changes. Taken together, our data suggest a novel activity-dependent mechanism for the observed plasticity in dopaminergic OB neuron populations.

Disclosures: **B. Grier:** None. **C. Cheetham:** None. **L. Belluscio:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: D.01. Chemical Senses

Support: Work supported by the NINDS Intramural Research Program

Title: Unraveling the olfactory bulb circuit: comparing the roles of perinatal and adult-born granule cells

Authors: *M. PALLOTTO, K. L. BRIGGMAN;
NIH / NINDS, Bethesda, MD

Abstract: In the olfactory bulb (OB) of mammals, inhibitory GABAergic interneurons, granule cells (GCs), regulate the activity of OB principal cells, specifically mitral (MCs) and tufted cells (TCs). MCs and TCs are functionally distinct and process different aspects of olfactory information, forming two different sub-circuits. In the mouse brain, GCs are produced from birth to adulthood. The role of GCs in the OB has been extensively investigated. However, whether the time of birth of these interneurons contributes differently to the inhibition of MC and TC output neurons is unknown. The aim of this work is to investigate whether adult-born and perinatal-born GCs have different functions in MCs -TCs OB sub-circuits. This question is key to understanding the intra-bulbar circuits and the specific role of adult-born cells. We used injections of adeno-associated viral vectors (AAV) at different time points, to visualize simultaneously both perinatal and adult-born GCs. The injections were performed at post-natal day 3 (p3) using a GFP-encoding AAV and at p50-60 using an RFP-AAV. Morphological analysis confirmed that adult-born GCs are located mainly in the inner GC layer, whereas p3 GCs are located mainly in the external part of the GC layer. Next, we used injection of viral vectors encoding calcium indicators (GCaMP6m and RCamP) and a two-photon microscope to monitor the excitability of perinatal and adult-born GCs. We found that both perinatal and adult-born GCs show calcium transients and respond to glutamate stimulation. Ongoing experiments use double-transgenic mice in which MCs and TCs express the light- activatable molecule ChR2. Using a custom two-photon microscope fitted with a DLP-based visual stimulator, we selectively optically stimulate TCs or MCs somata and record calcium responses in adult-born and peri-natal born GCs labeled with the calcium indicator. Lastly, we will collect large 3D electron microscope volumes from the same tissue to explore the connectivity among these and additional neuron types in the OB. These experiments will help us to investigate the specificity of connectivity between MCs, TCs and adult-born and perinatal born GCs, and therefore they may help us to better understand the role of adult neurogenesis.

Disclosures: **M. Pallotto:** None. **K.L. Briggman:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Title: Sexually-dimorphic neurophysiology in the main olfactory bulb of adult mice

Authors: *M. D. KASS, A. H. MOBERLY, J. P. MCGANN;
Psychology, Rutgers Univ., Piscataway, NJ

Abstract: In a number of species (including humans; e.g., Doty et al. 1985), females tend to outperform males when tested on a variety of olfactory behavioral paradigms. Despite these well-documented behavioral phenomena, the neurophysiological mechanisms by which sex influences olfaction have yet to be elucidated. To assess the effects of sex on early olfactory sensory processing, we performed *in vivo* widefield epifluorescence imaging in a line of gene-targeted mice expressing the exocytosis indicator synaptoHluorin (spH) under control of the olfactory marker protein (OMP) promoter in all mature olfactory sensory neurons (OSNs). Odorant-evoked spH signals in these mice linearly indicate neurotransmitter release from OSNs into olfactory bulb glomeruli, and thus permit the visualization of primary sensory odor representations. A bilateral cranial window was implanted above the dorsal olfactory bulbs of anesthetized, freely-breathing OMP-spH mice of both sexes, and optical spH signals were then acquired during the presentation of a panel of four monomolecular odorants, with each odorant in the panel being presented at three concentrations. We observed different patterns of OSN synaptic input to the olfactory bulbs of male and female mice. Most noticeably, odorant-evoked glomerular response maps in females contained a greater number of glomeruli receiving synaptic input than that observed in males. This difference was observed across a 4-fold range of odorant concentrations, and was independent of the odorant-response selectivity of individual glomeruli within each odor map. We also found that the most discriminative glomeruli, which only received OSN input from one out of the four odorants in our panel, had much larger response amplitudes in females than in males. Finally, we found that the latency to reach peak odorant-

evoked response amplitudes was shorter in females than in males, which is consistent with previous research (Shiao et al. 2012) showing higher expression levels of odorant binding proteins in olfactory epithelia from female mice than from male mice. This difference caused the overall map to evolve on a more rapid timescale in females compared to males, and could be a potential physiological sensory correlate of sexually-dimorphic olfactory abilities. In sum, it is possible that the sex-dependent differences in spatiotemporal primary odor representations that we observed here provide a neurophysiological substrate for the superior sense of smell seen in females.

Disclosures: **M.D. Kass:** None. **A.H. Moberly:** None. **J.P. McGann:** None.

Poster

719. Olfactory Coding: Second-Order Regions (Olfactory Bulb and Antennal Lobe)

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Support: R01DC011286

R01DC009994

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T15LM007056

T32NS007224

Title: The spatio-temporal input-output function of the olfactory bulb is modulated by respiratory cycle activity

Authors: *S. M. SHORT[1,2], T. S. MCTAVISH[1], T. M. MORSE[1], G. M. SHEPHERD[1], J. V. VERHAGEN[1,2];
[1]Neurobio., Yale Univ., New Haven, CT; [2]John B. Pierce Lab., New Haven, CT

Abstract: Spontaneous breath-evoked activity modulates mitral and tufted cell (M/TC) odorant responses. Odorants activate unique patterns of glomeruli. The strongest activated glomerulus functions as the primary glomerulus for driving a given M/TC. The responses reaching the M/TC somas backpropagate into the lateral dendrites, and are balanced by feedback and lateral

inhibition through granule cells. How the temporal and spatial aspects of glomerular input patterns influence the firing of a single M/TC, the main output of the olfactory bulb, is not currently understood, although distinct patterns of lateral inhibition are hypothesized to shape M/TC firing. Utilizing optogenetic stimulation techniques, we systematically analyze how specific temporal patterns of ORN glomerular input, which include combinations of primary and/or non-primary glomeruli, influence M/TC output activity. The spatial pattern, duration, frequency, and intensity of glomerular stimuli are manipulated optically across all breath phases using a custom-built Digital Micro-mirror Device, while *in vivo* extracellular recordings from single M/TCs are obtained in anesthetized OMP-ChR2 mice. This work further examines the roles of simultaneous activation of primary and non-primary glomeruli on M/TC activity. Findings highlight the importance of temporal coding and support the hypothesis that spontaneous activity associated with particular phases of respiration influences the efficacy of glomerular input patterns. Findings also indicate that spatial coding of glomerular input patterns reveals regions of excitatory and lateral inhibitory effects on M/TC firing. Additional work will continue to define the spatio-temporal map of the input-output function of the olfactory bulb.

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Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 720.01/FF3

Topic: D.01. Chemical Senses

Title: Nasal airflow entrains glomerulus-specific theta oscillations for phase odor coding

Authors: *R. IWATA[1], T. IMAI[1,2];
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Abstract: Odor information is represented by both intensity and timing of neuronal activity in the olfactory bulb, but origins and roles of the temporal pattern remain enigmatic. Here we addressed this issues using in-vivo two-photon calcium imaging of the mouse olfactory epithelium and olfactory bulb. We found that nasal airflow produces widespread responses in olfactory sensory neurons, and thereby entrains respiration-locked theta oscillations in mitral/tufted cells. Most glomeruli demonstrated the theta oscillations, but their oscillation

phases relative to the sniff cycles were glomerulus-specific. Changes in sniff speed or frequency changed activity intensity, but had minor effects on the relative phase. In contrast, odor stimuli generated odor- and glomerulus-specific phase shifts, indicating that phase information distinguishes odor responses from airflow responses. Notably, during odor sampling across multiple sniffs, the intensity of odor-evoked activity dynamically evolved over time; however, the phase code remained constant across sniffs. Thus, the phase code can more stably represent odor identity than the rate code. The airflow sensation by OSNs is essential for the phase odor coding, because the phasic representation of an odor was impaired under continuous airflow condition. Consistent with this observation, the phasic odor responses were less evident in the olfactory epithelium, suggesting that the oscillatory responses are mainly produced in the olfactory bulb circuits. Together, our results demonstrate that glomerular theta oscillations driven by the nasal airflow are the basis for reproducible perception of odor information. This phase coding may be a sampling mode-invariant and noise-resistant odor coding strategy.

Disclosures: R. Iwata: None. **T. Imai:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 720.02/FF4

Topic: D.01. Chemical Senses

Support: NIDCD, Senselab grant

NINDS grant NS11613

Title: Odor operators: formation, interaction, and computational properties of distributed synaptic clusters in the olfactory bulb

Authors: *M. MIGLIORE[1,2], F. CAVARRETTA[1,3], M. L. HINES[2], E. TULUMELLO[1], G. M. SHEPHERD[2];
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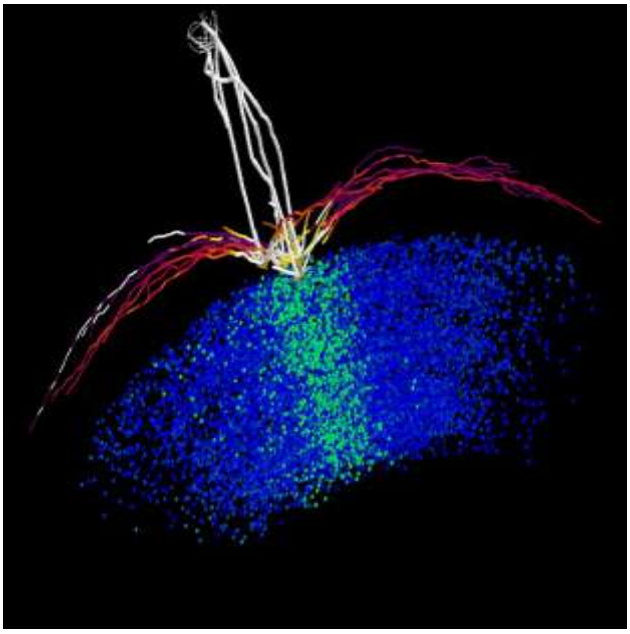
Abstract: The functional operations of the microcircuits that modulate the spatiotemporal dynamics of mitral cells in the olfactory bulb are difficult to explore experimentally. Recent experimental evidence suggests that odor processing before cortical action is organized in well-

defined, sparse, and segregated synaptic clusters. The observed columnar organization of these clusters can emerge from the interaction among odor inputs, action potential backpropagation in the mitral cell lateral dendrites, and dendrodendritic mitral-granule cell synapses. The feedback and lateral inhibitory action of the clusters can explain the experimentally-observed firing dynamics of mitral cells during sniffs of different odors. Here, using our latest full-scale 3D model of the olfactory bulb and natural odor inputs, we introduce the notion that the learning phase of any given odor generates an "odor operator", defined by the specific spatial configuration of the potentiated synaptic clusters that would be obtained in the absence of any prior network exposure to odors. In general, different operators may be composed of a number of clusters that may spatially overlap. During the lifetime of the mitral-granule cell network, operators can combine in more or less complex ways, according to the history of odor exposure. At any given time, the combination of the operators associated with the learned odors will determine how any odor input is perceived during a recognition phase. To explore the computational properties of odor operators, we generated and studied the operations of a relatively small set of them. Their relative spatial configuration included more or less overlapping synaptic clusters distributed in the dorsal part of the bulb. A typical example of an operator (composed of a single synaptic cluster) is shown in Fig.1. Odor operators can combine in predictable ways, and even simple operators can give rise to a number of complex operations, including selective spatial gating, non-linear summation properties, time-dependent actions during sniffs, and non-commutative operations.



Disclosures: **M. Migliore:** None. **F. Cavarretta:** None. **M.L. Hines:** None. **E. Tulumello:** None. **G.M. Shepherd:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Topic: D.01. Chemical Senses

Support: NIH/NIDCD Grant R01 DC012249

Title: A coupled-oscillator model of olfactory bulb gamma oscillations integrating both PING and STO mechanics

Authors: *G. LI, T. A. CLELAND;
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Abstract: Fast local field potential (LFP) oscillation in the gamma frequency band (30-80 Hz) is an emergent property of olfactory bulb (OB) circuitry and its underlying neuronal synchronization plays an important role in the coding and processing of olfactory information. However, the precise cellular and network mechanisms underlying coherent OB gamma oscillations remain elusive. The oscillogenic mechanism in OB has been modeled either as a synaptically-based architecture known as pyramidal/interneuron network gamma (PING; Davison et al. 2003; Bathellier et al. 2006) or as one based on the intrinsic subthreshold oscillations (STOs) of mitral cells (Brea et al. 2009). Each of these frameworks can explain some aspects of OB oscillogenesis, but each also has limitations when extended into more complex biophysical models incorporating glomerular computations, heterogeneous spike propagation delays among columns, and similar physiological factors. We present an explicitly two-dimensional (2D) biophysical OB network model including both glomerular and external plexiform layer computations and incorporating realistic distance-dependent spike propagation delays along mitral cell (MC) lateral dendrites. As in our previous OB model (Li and Cleland 2013), MCs exhibit intrinsic STOs and $GABA_A$-ergic synaptic transmission, which couples these intrinsic oscillators, is modeled as graded inhibition. A stable and broadly coherent oscillation emerges from the 2D network in the gamma range, coordinated by the interplay between MC spikes and GC subthreshold membrane depolarization. Simulations further indicate that MC STOs directly contribute to gamma oscillation, facilitating broad synchronization across the spatially extended network. Interestingly, our network model displays characteristics of both the PING and STO-dependent gamma models in that the oscillation frequency is tightly controlled by the decay time constant of the $GABA_A$-mediated inhibition, but also dependent on the uncoupled STO frequencies; for example, the $GABA_A$ decay time constant needs to be comparable to that of the intrinsic STOs for coherent oscillations to emerge. Furthermore, the

functional amplitude and frequency of MC membrane potential oscillations can be effectively regulated by periodic synaptic inhibition, facilitating the rise of strongly synchronized gamma oscillations. This 2D OB coupled-oscillator model integrates the strengths while resolving many of the limitations of the PING and STO-based mechanisms of gamma oscillation in olfactory bulb.

Disclosures: G. Li: None. **T.A. Cleland:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Topic: D.01. Chemical Senses

Support: NSF grant DMS-0719944

NSF RTG grant DMS-0636574

Title: Cortical control of network structure and stimulus discrimination in olfaction

Authors: *H. RIECKE, W. ADAMS, J. N. GRAHAM, J. C. DENNIS;
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Abstract: A striking feature of the olfactory system is the adult neurogenesis of granule cells, which leads to a persistent turn-over of this dominant interneuron population of the olfactory bulb. The turn-over endows the olfactory system with substantial structural plasticity; the function of this plasticity is, however, still poorly understood. We develop a minimal computational model that incorporates essential, experimentally observed qualitative features of the network formed by the olfactory bulb and the piriform cortex. As in our previous model of the olfactory bulb alone [1], the granule cells provide reciprocal inhibition to the mitral cells and their survival is governed by their activity. We now incorporate the fact that the granule cells also receive substantial excitatory centrifugal input from the principal cells of piriform cortex. These principal cells, which receive excitatory projections from the mitral cells, also make associational connections among themselves, endowing this cortical area with properties of an associational memory. We find that the resulting neurogenetic network evolution establishes a connectivity that allows the cortical cells to inhibit - via the granule cells - specific mitral cells. The resulting cortical control shapes odor representations in the bulb and can enhance the

discrimination of specific stimuli. Since the cortex receives also non-olfactory input, the representation of a given odor can - already in the olfactory bulb - reflect the context in which the animal experiences that odor, i.e. the bulbar representation may depend on information received from other sensory modalities or on the expectation of a reward. [1] S.F. Chow and H. Riecke, PLoS Comp Bio 8 (2012) e1002398

Disclosures: **H. Riecke:** None. **W. Adams:** None. **J.N. Graham:** None. **J.C. Dennis:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

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Program#/Poster#: 720.05/FF7

Topic: D.01. Chemical Senses

Title: Differential interactions between piriform cortex and olfactory bulb for orthonasal versus retronasal odors

Authors: *S. GAUTAM, W. SHEW;
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Abstract: Flavor perception shapes appetite and is, therefore, a fundamental governing factor of feeding behaviors and associated diseases such as obesity. Retronasal smell is an essential element of flavor, and refers to food volatiles that enter the nose via back of the oral cavity while eating. Human psychophysical and neuroimaging studies have established that orthonasal (inhaled through external nares) versus retronasal presentation of the same odor can evoke distinct perceptual experiences and activate differential brain regions. Recent optical imaging studies in rodent models have reported that such differences might be attributed, at least partly, to differences in the synaptic input to the olfactory bulb (OB) for ortho- versus retronasal stimulation. To further understand the sources of differences between ortho- and retronasal olfaction, here we compared the extracellular electrophysiological responses to odors at the level of Mitral/Tufted (M/T) cells in the OB and anterior Piriform Cortex (aPC). We further investigated if and how the cortico-bulbar interactions differ between the two modes of olfaction. Measurements were made with 32-channel electrode arrays in urethane-anesthetized double-tracheotomized rats. We found that unlike earlier imaging studies of synaptic input to OB, the average local field potential (LFP) and M/T cell spiking responses to retronasal odors in OB were not weaker than those to orthonasal odors. Silencing a portion of aPC by TTX selectively modulated the M/T cell spiking responses to retronasal odors but not to orthonasal odors.

Oscillatory LFP response to retro- and orthonasal odors, both at OB and aPC, were changed differently when GABA antagonist bicuculline was applied to OB. These results suggest that sources of perceptual differences between ortho- versus retronasal olfaction are likely to lie not only within the nose and OB but also in the cortex.

Disclosures: S. Gautam: None. **W. Shew:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Topic: D.01. Chemical Senses

Support: Whitehall foundation

PEW trusts Latin American Fellowship In The Biomedical Science

Title: Processing olfactory information of a single receptor type

Authors: *E. M. ARNEODO[1], K. PENIKIS[1], T. BOZZA[3], D. RINBERG[2];
[1]Physics, [2]Neurosci., New York Univ. Neurosci. Inst., New York City, NY; [3]Neurobio., Northwestern Univ., Evanston, IL

Abstract: Olfaction stands as an ideal model system for studying transformations of early sensory inputs due to the genetic tractability of the first-order neurons and the compactness of the first processing stages: the olfactory sensory neurons (OSNs) are only two synapses away from higher brain areas. The transformation imposed on the initial representation within the bulb remains elusive, because a) it is difficult to observe the output of a second order cell while knowing its specific input, b) it should be done in awake animal as network dynamics change drastically with wakefulness. We have developed an optogenetic technique to identify and record in awake animal from the second order neurons connected to the specific population of OSNs. In the mammalian olfactory bulb, axons of OSNs segregate by the genetic identity of their receptor type and converge to form glomeruli. Mitral/tufted (M/T) cells receive input from just one of these glomeruli and convey to the brain a transformation of this input. Using a transgenic mouse line expressing ChR2 in neurons of a single receptor type (M72), we can identify the M/T cells connected to the corresponding glomerulus by a consistent response following light stimulation of the OSNs. We recorded activity of these M/T cells in response to a battery of odors in awake,

head-fixed preparation with exquisite control of stimulus and sniffing time course. We chose odorants for which responses of M72 receptor neurons have been well characterized. We find that latency of M/T cell responses relative to inhalation onset conveys information about the odorant concentration and its affinity to the receptors.

Disclosures: E.M. Arneodo: None. **K. Penikis:** None. **D. Rinberg:** None. **T. Bozza:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Topic: D.01. Chemical Senses

Support: NSF Graduate Research Fellowship

Whitehall Foundation Grant

Title: Rapid and concentration tolerant odor identification via primacy coding

Authors: *C. WILSON, G. SERRANO, E. CHONG, A. RESULAJ, D. RINBERG;
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Abstract: Odors' perceptual qualities are largely maintained over a range of odor concentrations. This concentration invariance occurs despite changes in the olfactory sensory neuron (OSN) population representation. While OSN spike rates change, some evidence exists that relative latencies of these cells' response to odor are much less variable across concentrations. We propose a model of primacy coding in olfaction in which the small populations of OSNs that respond the most quickly to an odor define that odor's identity. While the absolute latency of activation changes with concentration, it is predicted that the same OSNs will be activated first across concentrations. Based on this model, odor identification should be possible using information transduced very quickly following the onset of odor sampling. To test this, we used an optogenetic-masking paradigm in mice to determine the time widow of sensory information processing relevant for odor-identity. Light stimulation of random population of OSNs expressing Channel Rhodopsin is used as masking stimuli presented at the specific defined times in the sniff cycle during odor discrimination paradigm. To ensure that a mouse solves this task based on odor identity and not intensity, we present odors at a range of concentrations interleaved throughout behavioral sessions. Our data show that only the first tens of milliseconds

at the beginning of the sniff cycle are required to discriminate between two odors. The length of unperturbed signal required is concentration dependent: this critical window dilates for discrimination of lower concentrations of the odors. These data suggest that odor-identification over a wide range of concentrations can be made from information transduced within the first fraction of a single sniff.

Disclosures: C. Wilson: None. **D. Rinberg:** None. **G. Serrano:** None. **A. Resulaj:** None. **E. Chong:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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DFG-SPP1392

BMBF

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MRC (MC_UP_1202/5)

ExcellenzCluster CellNetworks

Gottschalk foundation

Title: Independent control of gamma and theta activity by distinct interneuron networks in the olfactory bulb

Authors: *I. FUKUNAGA[1], J. T. HERB[1], M. KOLLO[1], E. S. BOYDEN[2], A. T. SCHAEFER[1]; [1]Neurophysiol., MRC, London, United Kingdom; [2]MIT, Media Lab and McGovern Institute Cambridge, MA

Abstract: Circuits in the brain possess a remarkable ability to orchestrate activities on different timescales, but how distinct circuits interact to sculpt diverse rhythms remains unresolved. The olfactory bulb (OB) is ideal for probing this question: inhibitory interneurons here are segregated

into distinct layers making selective manipulation possible, and, fast, gamma, and slow, theta rhythms coexist. Gamma oscillations are thought to originate in the reciprocal interactions between the principal neurons and granule cells (GCs) located in the deep layer. Mechanisms of theta rhythm regulations are less clear: classically, GCs have been attributed, but recent modelling studies suggest that circuits in the superficial layer, the glomerular layer (GL), underlie them. To dissect circuits that structure OB outputs on different timescales, we combined intracellular recordings *in vivo* with circuit-specific optogenetic interference both, in anaesthetized and awake animals. Adeno-associated virus for cre-recombinase dependent expression of archaerhodopsinT was injected into the centre and the superficial layer of the OB to target the GCL and GL, respectively, in Gad2-Cre and Vgat-Cre animals. In Vgat-Cre mice, intracellular recordings from GCs showed high infection rate of 85% or more (22/26 infected *in vitro*; 13/15 cells *in vivo*, in the awake) and light-evoked hyperpolarisation (>30 mV at 800 μm depth), strong enough to reliably silence evoked excitatory responses. Despite this, GC silencing in anaesthetized animals caused no change either in phase preferences or odour-evoked inhibition. In contrast, GL silencing had dramatic effects on both aspects of theta range activities, causing widespread phase-shifts (range; -2.68 to 1.25 radians, n = 23 cells) preferentially in mitral cells, and reduction of evoked inhibition (-3.60 ± 0.57 mV control vs. -1.97 ± 0.41 mV LED; $p < 0.005$, n = 7 cells). On the other hand, GCL silencing caused significant reduction of gamma power in the LFP (normalised gamma power = 0.92 ± 0.10 control vs. 0.67 ± 0.08 LED; n = 10 animals) as well as in the spike power spectrum (log(power/ .s) = -2.84 ± 0.48, control vs. -3.17 ± 0.23, LED; n = 10 cells). In addition, in awake, head-fixed animals, despite the possibility that GCs here might have greater influence on M/TC activities in general, GCL silencing did not affect the theta range M/TC activities. GCL silencing on the other hand significantly reduced gamma oscillations in the OB in the awake animals. The results suggest that dissociable inhibitory networks in the olfactory bulb control theta and gamma range activities separately, and that common mechanisms hold under different brain states.

Disclosures: **I. Fukunaga:** None. **J.T. Herb:** None. **M. Kollo:** None. **E.S. Boyden:** None. **A.T. Schaefer:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 720.09/FF11

Topic: D.01. Chemical Senses

Title: The effects of primacy on the encoding of odor identity

Authors: *A. KOULAKOV[1], D. RINBERG[2];
[1]Cold Spring Harbor Lab., COLD SPG HBR, NY; [2]NYU, New York, NY

Abstract: Behavioral studies in humans show that olfactory percepts can be embedded into a space of substantially small dimension (~6D). It is not clear how this low dimensionality can be reconciled with a large repertoire of the olfactory receptor types. At the same time, receptors display genetic sequences that are relatively stable across individuals. It is not clear how this stability is consistent with the redundancy pertinent to the random olfactory code. We propose the olfactory coding scheme that can shed light on some of these questions. We propose that an odorant identity is represented by a small number of olfactory receptor types that display the highest affinity to the odorant. The number of active olfactory receptor types that are sufficient to encode an odorant identity is denoted by the parameter p. Such a model based on receptor primacy can explain various observations pertaining to the olfactory code. First, we show that an evolutionary model for olfactory receptor types based on selection for primacy should reduce the dimensionality of olfactory perceptual space. The minimum dimensionality of perceptual space is described by d=p-1. This space can be tessellated by the p-simplexes representing various combinations of the highest affinity (primacy) receptors. Because the dimension of olfactory perceptual manifold is close to d=6, the number of receptor types sufficient to encode odorant identity is p<8. Second, random mutations in receptor genetic sequence can easily eliminate the receptor from the low-dimensional sensory manifold, reducing the probability of such mutations to be fixed. Low dimensionality of perceptual space, therefore, may be responsible for the stability of receptor sequences. Finally, primacy suggests connectivity patterns between consecutive stages of the olfactory processing. For insects in which the recurrent connectivity in the mushroom body (MB) is weak, we propose that the pattern of projections from antennal lobe (AL) implements the detection of the primacy combinations of p coactive receptor types with the highest affinity to various odorants. As a corollary, primacy model predicts specific high-order correlations in the AL-to-MB connectivity that result from the tessellation of insect perceptual space by p-simplexes.

Disclosures: A. Koulakov: None. **D. Rinberg:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 720.10/FF12

Topic: D.01. Chemical Senses

Title: Extracting principles of random maps from olfactory systems

Authors: S. SRINIVASAN[1], *C. F. STEVENS[2];
[1]Salk Institute; KIBM, UCSD, La Jolla, CA; [2]Salk Inst., La Jolla, CA

Abstract: Sensory perception shapes our view of the world. Its representation serves as input to circuitry that integrates various types of sensory information to perform computations that generate thought and action. Deciphering how sensory percepts are represented is crucial for understanding how this circuitry functions. We undertook this task by studying the olfactory system. Olfaction makes an excellent model system for three reasons. First, studies suggest that the piriform cortex - where most processing occurs - codes for odor percepts. Second, in contrast to other (topographic) sensory systems, odors activates a unique combination of glomeruli in the olfactory bulb, which in turn activate a random ensemble of piriform cortex neurons. This bulb-cortex transformation closely mimics information transfer within the field of random projections (or compressed sensing), providing a theoretical basis for predicting information transfer measures for any bulb-cortex pairing. Third, olfactory architectures are conserved across disparate phyla. We take advantage of this conservation to measure bulb and cortex components in three species of rodents and two carnivores, and test if indeed different olfactory architectures use the same principles to store and transfer information, even as they scale. Lessons learned from this study might also be helpful for understanding other types of brain circuitry which show evidence of random activation patterns, such as the hippocampus and cerebellum.

Disclosures: S. Srinivasan: None. **C.F. Stevens:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Topic: D.01. Chemical Senses

Support: Boehringer Ingelheim fonds PhD fellowship to SKJ

Title: Spontaneous activity governs olfactory representations in spatially organized habenular microcircuits

Authors: *S. K. JETTI, N. VENDRELL-LLOPIS, E. YAKSI;
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Abstract: The habenula (Hb) is an evolutionary highly conserved asymmetric brain region that connects the forebrain areas to the brainstem nuclei such as interpeduncular nucleus (IPN) and raphe. Genetic ablation of the dorsal habenula in zebrafish was reported to perturb experience-dependent fear response. It was also shown that Mitral Cells (MCs), the sole projection neurons of the Olfactory Bulb (OB), project their axons directly to the right Hb in an asymmetrical manner. However, it is unknown how the Hb processes olfactory information and how these computations relate to behavior. To address this question, we measured and compared the odor-evoked calcium signals in OB and Hb using two-photon microscope in juvenile elavl3:GCaMP5 zebrafish. We demonstrate that odor responses in the Hb are asymmetric and spatially organized despite the unorganized MCs inputs. Analyzing the spontaneous activity of Hb have revealed that spontaneous Hb activity is not random but highly structured into functionally and spatially organized clusters of neurons. We observed that the spatially organized Hb clusters are also preserved during olfactory stimulation, which govern the olfactory responses. Finally, we show that functional Hb clusters overlap with genetically defined Hb neurons that were reported to regulate experience-dependent fear. Our results demonstrate that the Hb is composed of functionally, spatially, and genetically distinct microcircuits that regulate different behavioral programs.

Disclosures: S.K. Jetti: None. **N. Vendrell-Llopis:** None. **E. Yaksi:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Topic: D.01. Chemical Senses

Support: National Institute on Deafness and Other Communication Disorders, National Institutes of Health (1 R01 DC012943)

Title: A model of background invariant odor recognition

Authors: *S. HANEY[1], D. SAHA[2], B. RAMAN[2], M. BAZHENOV[1];
[1]Univ. of California, Riverside, Riverside, CA; [2]Biomed. Engin., Washington Univ., St. Louis, MO

Abstract: Odors evoke complex neural responses that evolve over time. Temporal complexity of the response is compounded by various simultaneous slow processes including receptor adaptation. Further, natural environments contain a variety of stimuli including simultaneous presentations of multiple distinct odorants. *In vivo* recordings from locust (*Schistocerca americana)* antennae show that Olfactory Receptor Neuron (ORN) firing activity adapts at a wide range of rates depending on the odor presented. Recording from the locust antennal lobe shows that, in many but not all cases, transiently presented odors (foreground odors) in the presence of other persistently presented odors (background odors) will evoke similar neural responses as the foreground odor alone. Further, the similarity of these responses predicts the success rate in behavioral odor identification experiments. Here, we use a model of insect antennal lobe to investigate the role of receptor adaptation on odor discrimination of simultaneously presented odorants. By systematically altering ORN kinetics, we find that the adaptation of ORNs is necessary for the success of this task. Specifically, adaptation of the ORNs during background odor presentation is critical while foreground ORN adaptation is not. We propose a mechanism whereby adaptation of the background odor releases the system from the background odor attractor basin and allows it to flow toward the foreground odor attractor basin. Our study gives insight into how slow and adaptive neural processes can shape the response to a complex environment.

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Poster

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Topic: D.01. Chemical Senses

Support: NIH Grant DC012943

Title: Odor transformations through the layers of insect olfactory system

Authors: ***T. KEE**[1], P. SANDA[1], N. GUPTA[2], M. STOPFER[2], M. BAZHENOV[1];
[1]Univ. of California, Riverside, Riverside, CA; [2]NIH–NICHD, Bethesda, MD

Abstract: Neural representations of odors undergo multiple transformations as they progress through the olfactory system. Olfactory receptor neurons (ORNs) communicate odor information to projection neurons (PNs) and local neurons (LNs) in the antennal lobe (AL). PNs send signals

to the Kenyon cells (KCs) in the mushroom body (MB) and another population of neurons (LHNs) in the lateral horn (LH). KCs and LHNs respond very differently to odors. Among the huge population of KCs (50,000), only ~10% of cells respond to an individual odor, with each response consisting of 1-3 spikes. LHNs are far fewer in number, but most respond to any given odor, firing many spikes. To examine the odor information content in different structures of the insect brain, we designed a model of the AL-MB-LH network based on experimental recordings made *in vivo* in the locust (Schistocerca americana). When entire ensembles of neurons in the AL, MB, or LH were used for odor classification, the PN population showed very low error rates (<1%; 50% = chance) even given extremely similar odors (~99% overlap in ORN activation). KC and LHN populations showed higher error rates (up to 25%) given such very similar odor pairs, but classified less similar odors (<90% overlap) nearly perfectly. Odor classification improved with increasing stimulus duration: KC and LHN ensembles reached optimal discrimination within first 300-500 ms of odor-response. Using single PNs for classification yielded much lower accuracy (15-40% error rate). Interestingly, individual PNs showed greater accuracy either for high or low odor concentrations, but not for both. In contrast, individual LHNs (receiving input from 70% of PNs in our model) showed moderate discrimination accuracy (~25% error rate) for the entire range of odor concentrations and odor-pairs with varying degrees of similarity. We then applied principal component analysis (PCA) to spike trains across the PN ensemble to determine the minimal number of components sufficient for accurate odor discrimination. A single (first) PCA component provided very accurate discrimination (<1% error rate) even given very similar odors (~99% overlap), suggesting that a single downstream neuron could provide very accurate odor representations if it could sample the entire population of PNs. Further, for PNs, LHNs, and KCs, ensemble responses were always much more informative than single cell responses, despite the accumulation of noise along with odor information.

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Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Program#/Poster#: 720.14/FF16

Topic: D.01. Chemical Senses

Title: Serotonergic modulation of odor perception and olfactory bulb dynamics

Authors: *M. LEWIS[1], S. T. PEACE[1], G. LI[2], T. A. CLELAND[2], C. LINSTER[1];
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Abstract: Serotonergic afferents originating in both the dorsal (DRN) and median raphe nuclei (MRN) heavily innervate the rodent olfactory bulb, releasing 5-HT at both synaptic and non-synaptic sites. The effects of 5-HT are mediated through a variety of 5-HT receptors found in the bulb, including the 5-HT2 receptor class. At the behavioral level, recent work from our lab shows that the 5-HT2 receptor plays a critical role in the shaping of olfactory perception of near threshold odorants (10-3 Pa) in both a non-associative habituation and an associative two-alternative forced-choice digging task (2AFC). Interestingly, at higher odorant concentrations (10-2, 10-1 Pa), rats with bilateral bulbar infusions of the 5-HT2R antagonist cinanserin, were not impaired in 2AFC performance but exhibited significantly longer decisions times when making fine odor discriminations. To investigate the neural correlates of our behavioral results, we are investigating the effects of 5-HT on bulbar population activity and dynamics. We performed *in vitro* brain slice experiments using a 60-electrode planar microelectrode array (MEA; 200 um pitch, 30 um diameter, 30-50 kΩ impedance, 1.4 x 1.4 mm area) to record spike and local field activity under a variety of serotonergic conditions. In response to 5-HT, bulbar gamma oscillations are greatly increased in a dose-dependent manner suggesting that 5-HT may play a role in oscillatory network behavior. To explore the relationship between electrophysiological signature and perception, we are performing *in vivo* bulbar LFP recordings in conjunction with locally-administered serotonergic drugs during olfactory behaviors in rats. Lastly, we have constructed a biophysically-constrained multi-compartmental network model of serotonergic modulation in the olfactory bulb that is being used to investigate the role of 5-HT in bulbar physiology in conjunction with behavioral and neurophysiological experiments.

Disclosures: M. Lewis: None. **S.T. Peace:** None. **G. Li:** None. **T.A. Cleland:** None. **C. Linster:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 720.15/FF17

Topic: D.01. Chemical Senses

Support: NIH 1R01DC009948-01

Title: Locus Coeruleus stimulation reduces spontaneous activity and influences odor-evoked responses in the olfactory bulb of anesthetized rats

Authors: *L. C. MANELLA, C. LINSTER;
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Abstract: Behaviorally, noradrenaline (NA) decreases odor detection thresholds and enhances odor discrimination at low concentration odors. It is hypothesized that NA does this in part through increasing signal to noise ratio, particularly in the olfactory bulb. In this study, we use anesthetized rats to study how NA affects OB-dependent processing at low concentrations. To determine if NA affects baseline firing the OB, firing rates of mitral cells in the OB in response to a 60s 5Hz electrical stimulation in the LC (100uA, 100us duration) were measured. A majority of mitral cells show a significant reduction of firing rate (22/26 cells, with a median change of approximately 40%), which supports the idea that NA decreases background noise in the OB, although a few cells either do not change or increase their firing rates (4/26 cells). To determine how LC stimulation affects odor-evoked responses in the OB. Specifically, animals were presented with multiple 2s presentations of a mixture of odors at 0.1, 0.01 and 0.001 Pa odorants. Odor responsiveness before, during and 30, 60 and 90 after 5Hz stimulation of the LC (same as above) was tested. These results together show that LC activity influences signal to noise ratio in the OB, and thus influences near threshold odor responsiveness.

Disclosures: L.C. Manella: None. **C. Linster:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

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Topic: D.01. Chemical Senses

Support: Liu Memorial Award

Sigma Xi Research Grant

Cornell SAGE Fellowship

Title: Learning-dependent Egr-1 and c-Fos immediate-early gene expression in olfactory and valence circuits of the rat brain

Authors: *S. LUO, K. Y. KIM, L. TAN, K. V. GALL, M. EINHORN, T. A. CLELAND; Psychology, Cornell Univ., Ithaca, NY

Abstract: Appetitive and aversive consequences are intimately associated with the strength of learning and memory formation. However, the neuronal mechanisms underlying these two forms of memories may differ qualitatively, and are likely to involve multiple integrated networks. Unfortunately, direct comparison of the networks underlying appetitive and aversive learning has been difficult with conventional training paradigms due to their substantial procedural differences, which obscure whether observed differences in activity depend on the conditioning mode or are simply a reflection of the US modality and/or the different conditioned behaviors required for assessment. For example, an accumulating literature has shown that immediate-early gene (IEG) expression is intimately involved in synaptic plasticity and in various stages of learning and memory formation. While previous studies have examined Egr-1 and/or c-Fos expression following rewarded and fear conditioning, it often is difficult to assess IEG responses to valence per se, owing to the procedural differences which exist between appetitive and aversive conditioning paradigms. We developed an olfactory training protocol capable of evoking both forms of conditioning with minimal procedural differences, enabling outcomes to be directly compared using identical behavioral and physiological analyses. Using chronically implanted intra-oral cannulae, appetitive or aversive tastants (0.20% saccharin or 0.02 M quinine) were infused into rats' mouths and paired (or backward-paired) with an odor conditioned stimulus over 3 days such that training procedures differed only in the valence of the tastant. Rats displayed anticipatory behaviors (rapid mouth movements, tongue protrusions, gaping) in response to the odor CS predicting tastant infusions, indicating that rats recognized the differing valences of the tastants and learned the odor-tastant association. Preliminary data suggest that aversively-conditioned rats exhibit broader generalization to similar odorants than do the appetitive and control groups. We quantified Egr-1 and c-Fos expression in olfactory- and valence-associated brain regions following appetitive and aversive conditioning, including olfactory bulb layers, anterior olfactory nucleus subregions, rostral and caudal anterior and posterior piriform cortices, olfactory tubercle, subregions of the orbitofrontal cortex, and hippocampus. Such differences in IEG expression intensity and localization may help elucidate the pathways involved in appetitive and aversive associations, and enable study of the particular strengths of valence memories.

Disclosures: S. Luo: None. **K.Y. Kim:** None. **L. Tan:** None. **K.V. Gall:** None. **M. Einhorn:** None. **T.A. Cleland:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

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Topic: D.01. Chemical Senses

Support: NIH Training Grant T32GM007469

NIH Grant DC012249

Title: Coupled-oscillator properties of gamma-band field oscillations in olfactory bulb slices

Authors: *S. T. PEACE[1], B. JOHNSON[2], A. MOLNAR[2], T. A. CLELAND[3];
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Abstract: Coherent gamma oscillations (40-100 Hz) in the local field potential (LFP) during olfactory sampling are widely recorded across the rodent olfactory bulb (OB). Despite the prominence of these field oscillations, the network mechanisms that coordinate bulbar synchronization have yet to be fully described. The predominant theory of OB gamma oscillations is a pyramidal-interneuronal network gamma (PING) model that attributes oscillogenesis to the excitatory-inhibitory (E-I) synaptic interactions of the mitral cell (MC)-granule cell network in the external plexiform layer of the OB. Traditional PING oscillations necessarily depend on inhibitory synaptic activity and cannot be sustained without it. To elucidate the bulbar network that coordinates this synchronous activity, we recorded from 300 μm thick OB slices taken from young adult (P28-P42) transgenic mice expressing channelrhodopsin-2 (ChR2) under the control of the olfactory marker protein (OMP) promoter, hence limiting ChR2 expression to olfactory sensory neuron (OSN) axonal arbors within OB glomeruli. To enable large area recordings across the OB slice, we used a 60-electrode planar microelectrode array (MEA; 200 μm pitch, 30 μm diameter, 30-50 kΩ impedance, 1.4 x 1.4 mm area) to record spike and local field activity. Metabotropic glutamate receptor agonists (ACPD or DHPG) or a brief 4 Hz blue light stimulus were used to induce long-lasting (>5 min) gamma oscillations (20-50 Hz in slice) that are coherent up to 300 μm across the OB slice. Interestingly, the blockade of GABA(a) receptors in the OB slice (using BMI or gabazine) did not block these induced gamma oscillations, though it reduced their power, frequency, and limited the spatial extent of coherently oscillating regions to an area consistent with the field of a single glomerular column. In contrast, GABA(b) antagonists completely suppressed OB field oscillations, likely owing to a massive induced release of GABA from granule cells. Critically, gamma oscillations could be rescued from GABA(b)-dependent suppression by GABA(a) antagonists, indicating that the failure to block oscillations with GABA(a) antagonists was not due simply to insufficient blockade. These results suggest a coupled-oscillator model in which intrinsically oscillating intracolumnar networks are coupled by GABAergic synapses. Accordingly, we sought to block the BMI-resistant, putatively intracolumnar oscillations. In preliminary results, blockade of gap

junctions by carbenoxolone and inhibition of persistent sodium currents (which underlie MC subthreshold oscillations) by riluzole do not suffice to block BMI-resistant gamma oscillations.

Disclosures: **S.T. Peace:** None. **B. Johnson:** None. **A. Molnar:** None. **T.A. Cleland:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

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Program#/Poster#: 720.18/FF20

Topic: D.01. Chemical Senses

Support: Center for Behavioral Brain Science

Title: *In vivo* imaging of spatial patterns of neuronal activity in rat brain during predator odor exposure - A 99mTc-HMPAO-SPECT study

Authors: D. VINCENZ[1,2], K. WERNECKE[3,2], S. STORSBERG[4,2], *H. SCHEICH[1,2], W. D'HANIS[4,2], M. FENDT[3,2], J. GOLDSCHMIDT[1,2];
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Abstract: Predator odors such as carnivore urine represent a group of highly biologically-relevant chemosignals that enable perceiving prey animals to better assess predatory threats in their environment and to initiate appropriate defensive responses. Although the behavioral repertoire of anti-predatory responses (e.g. escape/avoidance, reduction in locomotor activity, risk assessment) has been described extensively, our knowledge about the neural circuitries mediating these innate fear responses is rather poor. We here used single-photon emission computed tomography (SPECT) imaging of regional cerebral blood flow (rCBF) for analyzing the spatial patterns of neuronal activity during fox urine exposure. We implanted rats with jugular vein catheters and intravenously injected, using a counterbalanced design in subsequent imaging sessions in the same animals, the blood flow tracer 99mTechnetium-hexamethylpropylenamine oxime (99mTcHMPAO) during fox urine exposure and during water exposure as control. 99mTc-HMPAO SPECT is similar in rationale to 18F-2-fluoro-2-deoxyglucose positron emission tomography (18F-FDG PET). After crossing the blood-brain barrier the lipophilic tracer 99mTcHMPAO is rapidly converted to a hydrophilic 99mTc-compound that remains trapped in the brain and shows no redistribution. Both tracers, 18F-FDG

and 99mTc-HMPAO, can be injected in awake behaving animals under stimulus-conditions and the stable radionuclide distributions can be read out in anesthetized animals after stimulation. We here used multi-pinhole SPECT for imaging the 99mTc-distributions in the rat brains after odor or water exposure. The blood flow images were intensity normalized, matched to a reference MR data set and analyzed using voxelwise statistics. We found highly significant increases and decreases in rCBF upon fox urine exposure in a number of brain structures. Prominent increases in rCBF were found in the ventromedial prefrontal cortex, the piriform cortex, the habenula and parts of the amygdala. rCBF decreased in the interpeduncular nucleus and the entorhinal cortex. Preliminary data suggest that these activation patterns change with different fear-coping behavioral strategies.

Disclosures: D. Vincenz: None. **K. Wernecke:** None. **S. Storsberg:** None. **H. Scheich:** None. **W. D'Hanis:** None. **M. Fendt:** None. **J. Goldschmidt:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

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Topic: D.01. Chemical Senses

Support: NIH Grant DC013090

NIH Grant DC009442

Title: Surprise alters primary sensory input to the brain

Authors: *L. A. CZARNECKI[1], D. J. TURKEL[1], A. H. MOBERLY[2], J. P. MCGANN[1]; [1]Psychology, [2]Rutgers Univ., Piscataway, NJ

Abstract: Mammals learn about regularities in their sensory environment, thus establishing expectations that can influence neural processing of sensory stimuli. Violations of these expectations can produce changes in neural and behavioral responses to sensory stimuli. Here we show that the violation of expectations about the presentation of a tone cue can influence sensory processing of odors as early as the first synapse in the olfactory system. We performed wide-field *in vivo* imaging from awake, head-fixed OMP-synaptopHluorin mice, in which odor-evoked neurotransmitter release from olfactory sensory neuron (OSN) axon terminals into the brain's olfactory bulb causes an increase in fluorescence that linearly reports the primary

olfactory input to the brain. In these mice, expectations were initially established by repeatedly presenting a light-tone-odor sequence and then violated by omitting the expected tone while presenting the light and odor as usual. Surprisingly, there was a suppression of odorant-evoked neurotransmitter release from OSNs during the surprising odorant presentation compared to the previous expected, tone-cued odorant presentation. This effect was not observed if mice were anesthetized or if the absence of the tone was unsurprising. Imaging of sniff-by-sniff calcium dynamics in OSN presynaptic terminals revealed that this suppression of activity is present on the very first inhalation of odorant during the surprising trial. The suppression of neurotransmitter release and presynaptic calcium influx suggested a GABA(B) receptor-mediated presynaptic inhibition, so we repeated the experiment in GAD2-GCaMP3 mice where we could visualize the activity of GAD65-expressing periglomerular interneurons. Consistent with this hypothesis, we observed an increase in GCaMP signals during the first inhalation of odorant on the surprising trial. Finally, we systemically blocked GABA(B) receptors with CGP35348 and observed that the drug removed a tonic presynaptic inhibition of OSN terminals, after which the surprising omission of an expected tone no longer had any effect on odorant-evoked neurotransmitter release. These experiments suggest that expectation and surprise can shape sensory processing as early as the primary sensory input into the brain.

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Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 720.20/FF22

Topic: D.01. Chemical Senses

Title: The role of BDNF-TrkB signaling in olfactory bulb-dependent olfactory memory

Authors: ***M. TONG**[1], T.-Y. P. KIM[1], E. L. GIBSON[2], R. SINGH[2], T. A. CLELAND[1]; [1]Psychology, [2]Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: Understanding the molecular mechanisms underlying short-term (STM) and long-term memory (LTM) has been a long-standing goal in neuroscience. Many putative forms of cellular LTM (including long-term plasticity and adult neurogenesis; Korte et al., 1995, PNAS; Scharfman et al., 2005, Exp Neurology) have been shown to depend on brain-derived neurotrophic factor (BDNF). One-trial fear-based memories additionally have been shown to rely

on specific temporal peaks of BDNF activity induced after learning (Bekinschtein et al., 2007, Neuron). However, the role(s) and timecourse of BDNF activity in multi-trial appetitive learning is unclear. We used olfactory bulb (OB)-dependent learning tasks to investigate the role of the BDNF-TrkB pathway in STM and LTM mechanisms. First, we examined whether odour-reward memories depend on TrkB-mediated BDNF activity in the OB. Adult mice were trained over several trials to learn an odour-reward association and memory was tested 2 or 48 hours later. Compared to controls, mice infused with OB infusions of the BDNF receptor antagonist, K252a, showed normal 2-hour memory, but impaired memory at 48 hours. This finding suggests that early activation of the BDNF-TrkB pathway is necessary for the consolidation of OB-dependent LTM, but not STM. Next, using an olfactory generalization task, we investigated whether LTM memory deficits on the odour-reward task are the result of disrupted odour memory representations. Finally, using RT-qPCR, we are mapping the timecourse of activity for bdnf and other plasticity-related proteins across multiple brain areas for incrementally-acquired olfactory memories.

Disclosures: **M. Tong:** None. **T.P. Kim:** None. **E.L. Gibson:** None. **R. Singh:** None. **T.A. Cleland:** None.

Poster

720. Olfactory Coding: Higher-Order Regions, Oscillations and Integrative Studies

Location: Halls A-C

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Program#/Poster#: 720.21/FF23

Topic: D.01. Chemical Senses

Title: Modeling beta oscillations in the coupled cortico-bulbar network

Authors: *B. L. OSINSKI[1,2], L. M. KAY[3,1];
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Abstract: Odors and other behaviors evoke oscillations in the local field potential (LFP) of the rat olfactory bulb (OB) and piriform cortex (PC) in gamma (60 - 100 Hz) and beta (20 - 30 Hz) frequency ranges. Beta oscillations increase in power as rats learn an olfactory discrimination task, while the converse is true for gamma oscillations. When centrifugal input onto granule cells in the OB is interrupted, beta power decreases and gamma power increases. To understand why cortical input to granule cells may be necessary for beta oscillations we developed a simple model of the coupled OB - PC network using single compartment leaky integrate and fire neurons with spiking synapses. Without feedback, the simulated LFP readily oscillates at gamma

frequencies, which are determined by the time constants of synaptic transmission. When feedback is introduced, the LFP oscillates at a beta band frequency, which scales with the transmission delays between OB and PC. Interestingly, adding a sinusoidal (respiratory) modulation of the input to the model tends to stabilize beta oscillations near a harmonic of the respiratory frequency over a wide range of transmission delay times. However, this simple model cannot explain the transition from gamma to beta that occurs shortly after a task-related odor is detected. To explain this sudden and highly reproducible transition we incorporate a two-compartment model of granule cells that includes both AMPA and NMDA type receptors on dendritic terminals. The added detail lets us explore several scenarios in which excitability of granule cells is integrated over time to trigger a transition from gamma to beta regimes.

Disclosures: B.L. Osinski: None. **L.M. Kay:** None.

Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: D.01. Chemical Senses

Support: R01 DC-003906 to DAW

Title: Dynamic cortical lateralization during olfactory discrimination learning

Authors: *Y. COHEN[1,2], D. PUTRINO[3,4], D. A. WILSON[1,2];
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Abstract: Bilateral cortical circuits are not necessarily symmetrical. Asymmetry, or cerebral lateralization, allows functional specialization of bilateral brain regions and has been described in humans for such diverse functions as perception, memory and emotion. There is also evidence for asymmetry in the human olfactory system, though evidence in non-human animal models is lacking. Here, we took advantage of the known changes in olfactory cortical local field potentials that occur over the course of odor discrimination training to test for functional asymmetry in piriform cortical activity during learning. The results demonstrate robust bilateral asymmetry in anterior piriform cortex activity that emerges during specific stages of odor discrimination learning, with a transient bias toward the left hemisphere. Furthermore, functional connectivity

(coherence) between the bilateral anterior piriform cortices is learning-, context- and event-dependent. Steady-state (many minutes) bilateral anterior piriform cortex coherence is enhanced as animals acquire competent performance in an odor discrimination task but is expressed only while they are in the learning context. No steady state change is observed in the home cage. Furthermore, event-dependent changes in anterior piriform cortex bilateral coherence emerge during specific stages of learning at specific behavioral phases of the discrimination trial. Together, the results provide the most detailed analysis of functional olfactory cortical asymmetry yet described in rodents.

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Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

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Topic: D.01. Chemical Senses

Support: NSF GFRP

Harvard University

Title: Odor and reward coding in the posterior piriform cortex

Authors: *D. MILLMAN, V. N. MURTHY;
Harvard Univ., Cambridge, MA

Abstract: In the rodent olfactory system, the posterior piriform cortex (PPC) is a major, but poorly understood, link between the representation of odors in primary olfactory cortex and the representation of behavioral goals and expectations in higher-order brain regions. Direct sensory input from the olfactory bulb (OB) reaches multiple cortical areas and the output of many of these areas converges on the PPC. Individual PPC neurons, in turn, send axonal projections that branch off to multiple known cognitive brain regions including orbitofrontal cortex, medial temporal lobe memory structures and limbic structures. The PPC also receives direct input from the orbitofrontal cortex and amygdala, which might allow associations between odors and their valence to be formed in the PPC itself. In order to understand the transformation of odor encoding at this bottleneck between sensory and cognitive brain regions, the activity of PPC neurons must be observed in animals during the performance of odor-driven tasks. We trained

mice to perform tasks while their heads were fixed in place and odors delivered directly to their nose with precise control over odor timing and concentration. The behavioral task involved learning the association between presented odors and whether or not a water reward will be available to the water-restricted mice. During the task, we recorded the spiking activity of individual neurons in the PPC with chronically-implanted, movable multi-tetrode drives. In addition, sniffing was monitored through a nasal cannula in order to address how odors are encoded within and across sniffs, which are the fundamental unit of olfactory experience. We found that individual PPC neurons were narrowly tuned to only a few odors in our panel and the responses of many neurons habituated strongly after the first sniff. Furthermore, individual neurons responded to odors without a strong influence from the associated reward valence. Finally, in ongoing experiments, we are recording from PPC as mice experience novel stimulus-reward associations to determine how these representations change in PPC through learning.

Disclosures: D. Millman: None. **V.N. Murthy:** None.

Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

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Support: NSF Grant No. DGE 0228243

Title: Mice lacking fragile x mental retardation protein have decreased olfactory sensitivity and intact spontaneous olfactory discrimination abilities

Authors: *A. SCHILIT NITENSON, E. E. STACKPOLE, J. R. FALLON, K. G. BATH; Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability (ID), resulting in cognitive impairments and altered sensory function. It results from an absence of the fragile X mental retardation protein (FMRP), an RNA-binding protein essential for proper synaptic plasticity and function. FMRP is expressed throughout the brain, but is highly enriched in the developing and adult olfactory bulb (OB). However, most work investigating the effects of disturbance in FMRP expression on sensory function have focused on the tactile domain with little attention to olfactory sensory function. To test the effect of loss of FMRP on olfactory discrimination ability as well as on olfactory sensitivity, we used a spontaneous

olfactory cross-habituation task at several odorant concentrations. We found that loss of FMRP led to a significant decrease in olfactory sensitivity in knockout mice compared with wildtype controls. When we controlled for differences in sensitivity, FMRP null mice showed no differences in their ability to spontaneously discriminate odorants. These data indicate that FMRP may play a significant role in olfactory sensitivity, with implications for understanding the role of the protein in sensory development and function.

Disclosures: A. Schilit Nitenson: None. **E.E. Stackpole:** None. **J.R. Fallon:** None. **K.G. Bath:** None.

Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

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Topic: D.01. Chemical Senses

Support: ELSC fellowship

Harvard University

Title: An olfactory cocktail party: Figure-ground segregation of odorants in rodents

Authors: *D. ROKNI[1], V. HEMMELDER[2], V. KAPOOR[2], V. N. MURTHY[2];
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Abstract: In odorant-rich environments, animals must be able to detect specific odorants of interest against variable backgrounds. However, several studies have suggested that both humans and rodents are very poor at analyzing the components of odorant mixtures, leading to the idea that olfaction is a synthetic sense in which mixtures are perceived holistically. We have developed a behavioral task to directly measure the ability of mice to perceive mixture components and found that mice can be easily trained to detect target odorants embedded in unpredictable and variable mixtures. Using OMP-GCaMP3 mice that express a Ca++ indicator in all olfactory receptor neurons, we were able to record the glomerular response patterns that are elicited by all individual odorants used in the task, and analyze the relationship between these response patterns and segregation difficulty. We found that: 1) Performance dropped steadily from 95% to 85% as the number of mixture components increased from 1 to 14 odorants. 2) Performance was lower when the target odorant and background odorants both contained the

tiglate functional group. And 3) The difficulty of segregating the target from the background was strongly dependent on the extent of overlap, but not similarity, between the glomerular representations of the target and the background odors. Our study indicates that the olfactory system has powerful analytic abilities that are constrained by the limits of combinatorial neural representation of odorants at the level of the olfactory receptors.

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Poster

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NIH-DC009910

T32MH096331

Title: Development of hedonics: Ontogeny of olfactory and limbic system circuits supporting maternal odor and predator odor responses in rats

Authors: *S. AL AIN[1,2,3], R. E. PERRY[1,2,3,4], K. MCSKY[3,5], R. SULLIVAN[1,2,3,4], D. WILSON[1,2,3,4];
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Abstract: Sensory systems must detect and discriminate environmental stimuli, but also tag information to that odor, including hedonic emotional content. Together, this provides the animal with sufficient information to emit the appropriate behavioral response. Tagging a sensory stimulus may be particularly important in the olfactory system, which is heavily and reciprocally connected to various limbic structures such as amygdala, orbitofrontal cortex and hippocampus. Indeed, convergence of olfactory pathways and limbic circuits are thought to enable the tagging of hedonics to the representation of the odor itself. Here we explore the development of hedonics, where the olfactory system is well-developed at birth, yet components of the limbic

system gradually functionally emerge (amygdala-postnatal PN10; prefrontal cortex (PFC)-PN15-18; hippocampus-PN18-23). We hypothesized that pups' changing odor hedonic response during development is a function of the expanding convergence of the limbic system with the olfactory system as pups transition to independence. Long Evans Rat pups at either PN8, PN15 or PN23 (weaning) were tested for hedonic responses to 10 monomolecular odorants to determine hedonic value as assessed by approach and avoidance (y-maze). Additional pups were used for neural assessment using C14 2-deoxyglucose autoradiograph (2-DG) and exposed to two preferred odors (maternal odor & acetophenone) and two avoided odors (predator odor & heptanal). Brains were processed and analyzed with Image J. The behavioral results show that responses to both aversive and preferred odors were relatively maintained across development, with some weakening of responses to preferred odors by PN23. The 2-DG result suggest that within the olfactory pathway, there is some convergence of response patterns to negative and positive odors, although distinctions occur with development. Hedonic value did not seem to alter with emergence of the amygdala into the neural circuit, although some changes in amygdala processing were noted. Finally, the PFC and hippocampal functional emergence into the limbic system was more closely associated with changes in hedonic value. These findings suggest that olfactory and limbic structures' neural network dynamics varied accross hedonic value and age and suggest a dynamic role for the limbic system in developmental changes in hedonic value.

Disclosures: S. Al ain: None. **R.E. Perry:** None. **K. Mcsky:** None. **R. Sullivan:** None. **D. Wilson:** None.

Poster

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Title: From molecules to motion: Assessing the responses of house crickets to plant volatiles using behavioral and electrophysiological paradigms

Authors: K. A. HUYNH[1], M. C. NESLUND[1], T. C. BAKER[2], *V. D. SHIELDS[1];
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Abstract: Olfaction plays an important role in the orientation and food source location for many animals including insects. Insects are capable of detecting and discriminating large numbers of odorants that differ in size, shape, and complexity. To gain insights into the mechanisms underlying odor-mediated orientation, it is necessary to study how odorants are detected, discriminated, and processed in the brain. The house cricket, *Acheta domesticus*, bears a pair of long antennae for the detection of diverse odorants by means of olfactory receptor cells (ORCs) residing in different types of cuticular sensory organs (sensilla). These sensilla act as the first level of environmental perception and are the crucial interface between the insect's outside world and its central nervous system. Odor molecules first reach the surface of these sensilla, perforated by many small pores, and find their way to the underlying sensory neurons where they bind to specific receptor sites. Here, the ORCs transduce the chemical stimuli into electrophysiological signals. This information is sent to the brain of the insect and provides neural input for higher order processing. Subsequently, this neural processing gives rise to behavioral orientation responses. In this study, we used a Y-tube olfactometer to screen a large number of host-plant-associated odorants, selected from a wide array of chemical classes, to determine which ones elicited positive (attractive) and negative (repellent) behavioral responses for both female and male crickets. In addition, we used an electroantennographic detection technique (EAG) to test the functional relevance of these odorants. We found some volatiles elicited strong EAG responses, while others evoked medium to weak responses. The results of these combined research approaches contribute to our knowledge of important plant odorants necessary for insect-plant interactions.

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Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

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Title: Arc catFISH visualization of odor engrams in the rat pup

Authors: *A. GHEIDI[1], A. SHAKHAWAT[1], Q. HOU[1], D. F. MARRONE[3], C. W. HARLEY[2], Q. YUAN[1];
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Abstract: The brain network changes that accompany a mnemonic event are poorly understood. An early Hebbian theory suggests that network ensembles may become stabilized by learning. Early odor preference learning via a single naris in week-old rat pups permits testing of odor memory processes within the same animal. Due to the lack of mature anterior commissural projections, odor learning can be confined to one olfactory hemisphere through single naris occlusion during training. Our previous work using *ex vivo* calcium imaging (Fontaine et al., 2013) suggested an enhanced odor representation in the anterior piriform cortex following learning - the threshold for pyramidal cells to respond with action-potential dependent calcium transients was lowered in the learned hemisphere. This suggests early odor learning may strengthen previously weakly responsive cells through synaptic plasticity so that those cells are recruited more reliably to the conditioned odor input. Here we directly tested this stabilization hypothesis by assessing activation of the immediate-early gene Arc using cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH), which permits visualization of activated neurons from two different time points in rat pups. Rat pups underwent multi-day odor + stroking or odor only training with single naris occluded. Twenty-four hours following the last training, pups were subjected to two peppermint episodes separated by 25 min. After the 2nd odor exposure, brains were extracted for Arc catFISH. Preliminary results show that the proportion of anterior piriform cortex cells that transcribed Arc in response to both peppermint episodes were greater in the odor + stroking training condition (paired t-test between occluded and spared hemisphere) relative to the odor only condition . However, the total number of Arc+ cells responding to peppermint in the learning and non-learning groups did not change, the percentage of Arc+ cells that responded to peppermint twice increased significantly. This is consistent with our hypothesis of a stronger representation from previously weak odor encoding cells following odor associative learning. The anterior piriform cortex recruits the same neurons more reliably for a given rewarded odor stimulus, but does not change the net amount of activity following learning. Currently, we are investigating the odor representations in the olfactory bulbs from the same animals. Together, this study will shed light on how rewarded odor is represented in the olfactory system in rat pups.

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Poster

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Title: Temporally coherent ensemble neural activity in an olfactory system elicits predictable behavioral response in an odor recognition task

Authors: D. SAHA, C. LI, S. PETERSON, W. PADOVANO, N. KATTA, *B. RAMAN; Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Coordinated spiking activities across a population of neurons have been observed at a variety of temporal and spatial resolutions and in different neural circuits. Such synchronous neural signals are thought to provide a substrate to integrate pieces of information individually encoded by neurons, and are therefore considered important for neural computations. Whether the neural synchrony is a fundamental response feature necessary for stimulus recognition and what are the behavioral consequences of disrupting population-level response synchrony are not known. Here, we investigated these issues in the insect olfactory system. We found that most olfactory stimuli evoked a highly coordinated response in the neural circuit downstream to the sensory neurons. The observed neural synchrony was disrupted when a persistent preceding stimulus that evoked an overlapping response was presented to alter the stimulus history. As a result, the overall response intensity was significantly diminished but odor identity was still robustly maintained. Notably, using a quantitative behavioral assay, we found that the temporal epochs during which neural synchrony were observed also corresponded to the time segments when a predictable behavioral response was elicited by the conditioned stimuli. Hence, our results reveal an important role for coherent neural activity in this olfactory system. Furthermore, our work reveals how neural networks employing a combinatorial coding scheme could use disruption in neural synchrony as an elegant gating mechanism to selectively deemphasize certain succeeding sensory stimuli based on their novelty.

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Poster

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Title: The synchronized neural activity of olfactory neuron AWC with the head movement in Caenorhabditis elegans

Authors: *M. MAKINO, H. SHIDARA, K. HOTTA, K. OKA;
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Abstract: Caenorhabditis elegans is known to sense odors and show taxis toward the source of favorite odor by detecting its concentration gradient. When the concentration gradient is vertical to the progress direction, the worm follows the weathervane strategy; the direction of worm progress bends gradually towards the higher concentration. However, little is known how exactly worms can discriminate the concentration of odor and change their head turning behavior in regard to the response of olfactory neurons. Furthermore, there is no precedent study to investigate the relation between worms' sensitivity of the concentration change and the weathervane strategy at the neural system level. We, therefore, investigated the quantitative relation between precise odor stimulation and the response of worms by head turning assays and the Ca2+ imaging. We used a head turning device for investigating the fine head movement, and demonstrated the olfactory sensitivity of individual worms. We applied laminar flow of isoamyl alcohol (IAA) with different concentration to the worm head and evaluated its behavior. Worms frequently turn their heads toward the higher concentration of the IAA flow, and we found that worms could distinguish ~ 10% difference of IAA concentration. Next, we monitored the Ca2+ change in the AWC olfactory neuron when worms sense the concentration change of the odor. We fixed transgenic worms expressed GEM-GECO in AWC in the microfluidic device, and applied diluted IAA (0.5 % ~ 40 % higher than control stimuli as the test stimuli and then 10-4 diluted IAA as the control stimuli) to worms. When the concentration of IAA changed lower, the Ca2+ change occurred in AWC (off response). We found that AWC can detect only 0.5 % difference of IAA concentration. Furthermore, we visualized the head turning and the Ca2+ change in AWC, simultaneously. We made laminar flow of IAA and water by using the head turning device, set a transgenic worm (str-2::GEM-GECO) in it, calculated its head curvature and monitored the Ca2+ change in AWC. We succeeded to observe synchronized AWC activities with worms' head moving. The Ca2+ increase in AWC occurred quickly when worms

shake their heads from IAA side to water side, but not occurred from water side to IAA side. From these assays, we concluded that *C. elegans* has highly sensitive olfactory neuron and that AWC's response corresponds with head turning behavior. AWC is activated when worms turn their heads from odor side to non-odor side, and this result suggests that AWC's response could be required to determine the direction of movement and affect head turning behavior in weathervane strategy.

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Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

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Topic: D.01. Chemical Senses

Support: NIDCD DC006666

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Title: Retronasal but not orthonasal presentation of odors is sufficient for learning in an olfactory preference task

Authors: *M. BLANKENSHIP, J. MAIER, D. KATZ;
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Abstract: The human olfactory system can be separated into two main 'modes' of stimulant experience: orthonasal delivery of odorants, in which odorants in the environment are sampled via inward direction of sniff, and retronasal delivery, where odorants present in the mouth and nasal cavity are sampled via outward sniff direction. Understanding the sort of olfactory information an organism gleans through these two modes has been implicated in our understanding of flavor perception and odor learning. In this work, we ask whether the mode of odor stimulant delivery_retronasally via installation of inter oral cannulae or orthonasally via odorant stream delivered through a nose poke--affects the ability to learn an odor-taste pairing in a preference learning paradigm in rats. Animals are trained to associate one of two orthonasally or retronasally delivered odors with a sweetened water reward versus a water reward alone, using a two nose poke olfactometer system. Following training, preference for the sweet-associated odor is assessed by measuring the number of nose pokes initiating delivery of the sweet

associated odor, compared to the odor paired with water alone. We found that retronasally presenting odorants during training and testing is sufficient to produce a preference for a sweet-associated odor, while orthonasal presentation during training and testing is not. This experiment indicates that the "outward sniff" mode of olfaction is required for learning associations between tastes and smells, which is consistent with its role in flavor perception as it samples from odors originating from the mouth and nasal cavity. We also found that a preference learned through retronasal presentation of odors is not expressed if animals are tested in the orthonasal olfactory mode. Taken together, these findings suggest that retronasal and orthonasal olfaction may not provide information of equivalent value for the formation of taste associations.

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Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

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Support: Harvard University

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Title: Figure-ground segregation of odorants in rodents: A simple model linking imaging and behavior

Authors: *A. MATHIS, D. ROKNI, V. HEMMELDER, V. KAPOOR, V. N. MURTHY; Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: The segmentation of complex sensory scenes into its components is a crucial ability for most sensory modalities, including olfaction. Behavioral results from our lab demonstrated that mice can be trained to detect the presence of a target odorant embedded in unpredictable and variable mixtures of up to 14 odorants. Performance is near perfect (95%) for stimuli composed of just one odorant and drops to 85% in mixtures of 14 odorant (see poster by Rokni et al.). We asked how difficult it is, in principle, to detect the presence of the target odor in a mixture of odors given neuronal recordings at the first stage of the olfactory processing path - the glomerular layer in the olfactory bulb. Olfactory receptor neurons (ORN) are broadly tuned to multiple odors and any given odor typically evokes responses in multiple ORNs leading to a

distributed representation. Given the large number of possible mixtures, we imaged glomerular activity maps individually for the 16 odors used in the behavioral study (we got 50-70 glomeruli per map) and approximated the responses to mixtures as linear responses. Based on these maps, we trained a classifier in a supervised way to detect the target odor. Specifically we used a logistic regression model with a sparse weight prior. In the case without neuronal noise, we find that such a classifier can almost perfectly classify (> 99% correct) when trained on around 10% of the trials. Typically the classifier is based on only 5-10 glomeruli. The performance of a classifier degrades gracefully if trial-to-trial variability is added to the glomerular maps and if saturation of the summed responses in included. Thus, despite the distributed representation at the first stage, a simple read-out neuron could already respond in a target specific manner. Currently we are exploring what strategies mice use while learning the task and compare it to the evolution of learning along trials for classifiers.

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Poster

721. Olfaction: Behavior Perception and Relationship to Neurophysiology

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Topic: D.01. Chemical Senses

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CIHR

Title: Arc catFISH visualization of pyramidal cell ensembles in the anterior piriform cortex following odour discrimination learning

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Abstract: Research is shedding light on how experience alters neuronal ensemble dynamics in the cortex. Here we addressed this question by assessing activation of the immediate early gene, Arc, using the cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) technique, which permits visualization of activated neurons from two

different time points in adult rats. We investigated how pyramidal cell ensembles in the anterior piriform cortex support and represent odors both in naïve rats, and in rats that underwent odor discrimination training. First, we demonstrated that pyramidal cells in naïve rats express Arc in an input-specific manner: more pyramidal cells transcribed Arc twice (Arc observed in both nuclei and cytoplasm) to the same odour (peppermint) presented twice (25 min separation) than those exposed to two different odours at those times. We next trained water-deprived rats in a go/no-go odour discrimination task using peppermint as a rewarded odour (S+) and vanillin as a non-rewarded odor (S-). Following successful odour discrimination, the cell ensemble of the rewarded odour became more reliable and sharper. We then used a mixture of peppermint and vanillin as the S+ and amyl acetate as the S-. Following discrimination training on the mixture rats responded positively to either peppermint or vanillin alone. Visualization of the ensemble for each component of the odour mixture revealed higher overlap between the ensembles than was found in control animals with random pairings of odor and water reward. This result is consistent with the hypothesis that pyramidal cells in the anterior piriform cortex are capable of producing engrams for the components of an odor mixture that support successful behaviour with degraded input (pattern completion). Finally, two very similar odour mixtures (1-heptanol and 1-octanol; S+: 53%/47%; S-: 55%/45%) were used for the odor discrimination problem, which required significantly more trials for successful discrimination than had been necessary for more dissimilar odours (peppermint and vanillin). The difficult odour discrimination induced a de-correlation of the two odour ensembles for each mixture (pattern separation), i.e., less overlap was seen for ensembles in the trained rats than in random controls. Interestingly, this overlap change did not occur with the easier discrimination. These results demonstrate that the anterior piriform cortex in adult rats responds dynamically to reward contingencies by modifying odor ensemble representations to support adaptive behavioural outcomes.

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Poster

722. Mechanoreceptors and Cochlea

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Title: Transcriptome profiling with RNA-Seq uncovers expression of candidate Xenopus genes for Alzheimer's disease and hearing loss

Authors: *D. RAMIREZ-GORDILLO, E. E. SERRANO;
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Abstract: Recent literature suggests an intriguing correlation between hearing loss and Alzheimer's (Lin et al., 2011). Both disorders are more prevalent in aging populations and both disorders have genetic underpinnings that are reported in the Online Inheritance in Man (OMIM) database. Here we present results from our continued efforts to determine whether the amphibian, Xenopus, can serve as a useful model for investigations of the linkages between these disorders. Previously we mined the OMIM database to identify genes linking hearing loss and Alzheimer's (Ramirez-Gordillo et al., 2013, SFN Abstract 334.15). In this study we extended these findings by determining the expression of these candidate genes in Xenopus inner ear and brain transcriptomes. Total RNA extracted from X. laevis adult inner ear and brain was sequenced with Illumina HiSEQ 2000 technology at the MIT BioMicro Center. Several programs in the Galaxy open source platform were used to identify the expression of the candidate genes in Xenopus. TopHat was used to align the sequences to the X. tropicalis reference genome, and Cufflinks was used to assemble the transcript for expression analysis. Analysis showed that several OMIM genes previously identified as associated with hearing loss and with Alzheimer's yielded expression values in the RNA-Seq data for both Xenopus inner ear and brain. Furthermore, several of the candidate Alzheimer's genes identified using STRING, including APP, PSEN1, and PSEN2, were present in the RNA-Seq data for Xenopus brain and/or inner ear. Results of this investigation confirm that OMIM genes linking hearing loss and Alzheimer's are expressed in Xenopus inner ear and brain. Our findings suggest that Xenopus may afford unique opportunities for studies of Alzheimer's disease and for exploring linkages between hearing loss and Alzheimer's.

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Poster

722. Mechanoreceptors and Cochlea

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Topic: D.02. Auditory

Title: Maintenance of stereocillia and apical junctional complexes by Cdc42 in cochlear hair cells

Authors: *Y. NINOYU[1], T. UEYAMA[1], H. SAKAGUCHI[2], T. NAKAMURA[1], A. GOTO[3], S. MORIOKA[1], B. FRITZSCH[4], Y. HISA[2], M. MATSUDA[3], A. AIBA[5], N. SAITO[1];
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Abstract: The sound-evoked mechanoelectorical transduction (MET) is a key phenomenon of hearing and it takes place in cochlear hair cells (HCs) via coordinated movement of stereocillia. Stereocillia, which are actin-based apical protrusions, are known as exquisitely organized microvilli composed of hundreds of parallel actin filaments. The length and shape of stereocillia are determined by precisely and tightly regulated actin turnover; however, the mechanism has not been fully understood. Here we focused on the role of Cdc42, an actin regulatory Rho-GTPase, in inner ear HCs. We employed HC-specific conditional knockout mice to analyze the role of Cdc42 in HCs. Hearing function was assessed by auditory brainstem response (ABR). Localization and activation of Cdc42 in HCs were examined using adenovirus-encoded GFP-Cdc42 and transgenic mice expressing a Cdc42 fluorescence resonance energy transfer (FRET) biosensor (Cdc42-FRET biosensor mice). Using MDCK cells with stable knockdown (KD) of Cdc42 as an in-vitro model of HCs, formation/morphology of microvilli and actin regulatory signaling were studied. Ultrastructural morphology was analyzed by scanning and transmission electron microscopy. Cochlear HCs of the Cdc42-KO mice normally developed but progressively degenerated after maturation, resulting in progressive hearing loss particularly at high frequencies. HC degeneration was firstly observed as deformity or depletion of stereocilia, accompanied with thinning and waving circumferential actin belt. Adenovirus-encoded GFP-Cdc42 presence and activation at stereociliary membranes and AJCs. The morphological phenotypes observed in Cdc42-KO cochlear HCs were reproduced in Cdc42-KD MDCK cells. Down regulated actin turnover presented by the enhanced levels of phosphorylated cofilin in Cdc42-KD MDCK cells was confirmed in Cdc42-KO cochlear HCs. Thus, Cdc42 plays pivotal roles in the maintenance rather than the development of stable actin structures, including stereocillia, microvilli and AJCs through elaborate tuning of actin turnover, and maintains function and viability of cochlear HCs.

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Poster

722. Mechanoreceptors and Cochlea

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Title: Simultaneous declines in NKCC1 and Na, K-ATPase, but not Kir4.1 and KCNQ1/KCNE1, are found in the cochlear lateral wall of CBA/CaJ mice with age-related hearing loss

Authors: B. DING[1], X. ZHU[2], R. D. FRISINA[2], *D. FRISINA, SR[3], J. P. WALTON[1]; [1]Communication Sci. & Disorders Dept., [2]Chem. & Biomed. Engin. Dept., [3]Univ. of South Florida, Tampa, FL

Abstract: The cochlea is a unique bodily organ with the largest trans-epithelial voltage and a very high extracellular K+ concentration, comprising the endocochlear potential (EP). Age-related stria vascularis degeneration and EP declines are considered hallmarks of age-related hearing loss, termed as metabolic presbycusis. Maintenance of stria function and a normal EP requires coordinated cellular mechanisms involving ion channel, exchanges and pumps, within the stria and adjacent spiral ligament. In general, the basic K+ regulators of the EP within the stria are Kir4.1 K+ channels localized in intermediate cells, Na, K-ATPase, the ion exchanger NKCC1, and KCNQ1/KCNE1 K+ channels expressed in marginal cells. Artificially reducing the kinetics or expression of these molecules, such as gene knock-outs, can lead to profound hearing loss. So, there is a need to explore age-related alterations of these essential elements of cochlear K+ secretion in animal models to understand the cellular and molecular mechanisms of presbycusis. Therefore, we compared the gene and protein expressions of NKCC1, Na, K-ATPase, Kir4.1 and KCNQ1/KCNE1 in the cochlear lateral wall of CBA/CaJ mice of different ages (3, 24 and 35 mon). We found that the protein expressions of NKCC1 and Na, K-ATPase were decreased in the lateral walls of old animals (24 and 35 mon) compared to the young adult group, and the 35 mon mice showed the greatest decline. But only the mRNA level of Na, K-ATPase presented a concomitant reduction with age, suggesting that an age-related post-translation modification of NKCC1 may exist in the aging cochlea. No age-related changes of protein and mRNA expressions for Kir 4.1 and KCNQ1/KCNE1 were observed. Using a micro-colorimetric assay measuring free inorganic phosphate (pi) from ATP, and immunoprecipitation of homogenized cochlear samples with NKCC1 and phospho-threonine antibodies, decreased Na, K-ATPase activity and NKCC1 phosphorylation [upon further determination for the

residue(s) of phosphorylation] were observed in aged cochlear lateral walls compared to young adult animals. These findings are consistent with the peripheral auditory deficits of elevated ABR thresholds and declined DPOAE amplitude in aged CBA/CaJ mice. Overall, these results demonstrate important roles of Na, K-ATPase and NKCC1 in metabolic age-related hearing loss, paving the way for future interventional targets to prevent or slow down the progression of presbycusis. Supported by NIH Nat. Inst. on Aging Grant PO1 AG009524.

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Poster

722. Mechanoreceptors and Cochlea

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Title: Targeted deletion of oncomodulin leads to changes in protein expression and efferent innervation

Authors: *D. D. SIMMONS, M. DAI, A. HORNAK;
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Abstract: Oncomodulin (Ocm) is a member of the parvalbumin family of calcium binding proteins. Within the cochlea, Ocm is almost exclusively found in the outer hair cells (OHCs). We investigated prestin expression in Ocm null mutants. We compared prestin expression with alpha-parvalbumin (αPV) and changes in OHC efferent innervation. Targeted deletion of Ocm results in progressive hearing loss beginning at 2 months with significantly elevated threshold shifts. In Ocm mutants, we found that prestin immunoreactivity (-ir) develops normally through the postnatal period. Beginning at 2 months, Ocm null mutants demonstrate increasing amounts of hair cell loss in basal high frequency regions. However, we confirmed that the most dramatic changes in prestin-ir occurred at 3 months. In general, prestin-ir in surviving hair cells was much more intense than in wild type controls consistent with either an increase in prestin expression or a decrease in OHC volume. Mutant animals exhibit a basal to apical prestin-ir intensity gradient, a greater number of prestin-labeled plaques, and regions of prestin-labeled OHC fragments. Additionally, prestin-labeled OHCs are shorter especially in apical regions. In mice, both mutants and wild-type controls express αPV-ir up until the first postnatal month. We did not observe any changes in αPV-ir in mutants compared to wild type controls. However, Ocm null mutants show less αPV gene expression. In mutants, efferent terminals were found on intact prestin-labeled OHCs and appeared normal at 1 month. In 2 and 3 month old Ocm mutant mice, efferent terminals are not present where prestin labeling is absent or diminished. Efferent terminals show significant remodeling in areas of the cochlea where prestin is absent. In general, the OHC efferent terminals in mutants were smaller in size than wild-type controls. The loss of efferent terminals coincides with changes in prestin expression. These data suggest that targeted deletion of Ocm leads to altered expression of proteins and that the elevation of hearing thresholds may be a consequence of altered prestin expression.

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Title: Cisplatin treatment modulates the cochlear expression of LMO4 downstream targets

Authors: *S. JAMESDANIEL;
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Abstract: Hearing loss is one of the major side effects of the most frequently used chemotherapeutic drug cisplatin. Although considerable progress has been made in delineating the mechanisms underlying cisplatin-induced ototoxicity, the components of apoptotic pathways that facilitate cochlear apoptosis are yet to be fully characterized. We recently identified LMO4 as a potential mediator of cochlear apoptosis and a plausible target in cisplatin ototoxicity. This study aims to evaluate the cisplatin-induced modulation of known LMO4 interactomes and downstream targets in ototoxicity. Custom-made gene arrays (SABiosciences) were used to investigate the cochlear mRNA levels of at least 10 genes that are known binding partners or targets of LMO4, in Wistar rats treated with 16 mg/kg cisplatin. Since nitration of LMO4 was identified as a critical factor in cisplatin-induced ototoxicity, antioxidant Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is an inhibitor of peroxynitrite and an otoprotective agent, was used to inhibit cochlear protein nitration. qRT-PCR analysis of the cochlear expression of LMO4 interactomes, 3 days post cisplatin treatment, indicated that cisplatin-induced up/down regulation of 5 genes was attenuated by co-treatment with Trolox. More importantly, the cochlear expression of ESR1 was significantly up-regulated by cisplatin treatment, while the expression of STAT3 and JAK1 was down-regulated. Co-treatment with Trolox attenuated their cisplatin-induced modulation in the cochlea. Consistent with the changes observed at the gene level, immunoblots with anti-STAT3 indicated that cisplatin-induced decrease in cochlear protein levels were attenuated by Trolox co-treatment. LMO4 acts as a scaffold to stabilize gp130 complex, which facilitates the phosphorylation and activation of JAK1 and leads to the recruitment and phosphorylation of STAT3. Hence, the results of this study suggest that cisplatin-induced decreases in the cochlear expression of LMO4 upon nitration, and associated modulation in the cochlear expression of its binding partners ESR1 and JAK1, probably facilitates the repression of STAT3, a downstream target of LMO4 implicated in drug mediated apoptosis. Collectively, these findings indicate the potential role of JAK/STAT transcriptional machinery in mediating LMO4 protein signaling in cisplatin-induced ototoxicity.

Disclosures: S. Jamesdaniel: None.

Poster

722. Mechanoreceptors and Cochlea

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 722.06/GG9

Topic: D.02. Auditory

Support: Office of Naval Research,Grant #N000141310569.

National Center for Research Resources-Health Grant #S10RR027716

Title: Survival of regenerated hair cells in the neonatal mouse cochlea

Authors: *B. C. COX, S. KARMARKAR;
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Abstract: Background: Previous studies have shown that hair cell (HC) damage in the neonatal mouse cochlea results in spontaneous HC regeneration *in vivo* with new HCs derived from supporting cells. However, most regenerated HCs died within 1-2 weeks, with only a few newly formed HCs surviving to postnatal day (P) 15 (Cox et al., 2014 Development). The current study uses fate-mapping techniques to distinguish between regenerated HCs and surviving HCs (that were not killed by the initial damage protocol) to quantify the total number of regenerated HCs that survive to P15 or later ages, as well as to investigate the expression of Pou4f3, a gene linked to HC survival (Erkman et al. 1996 Nature), and Prox1, a gene that has been shown to cause cell death when ectopically expressed in HCs (Kirjavainen et al. 2008 Dev Biol). We hypothesize that after HC damage, the majority of regenerated HCs at P4 lack expression of Pou4f3, but do express Prox1; while at P15 this pattern is reversed with the few regenerated HCs that survive expressing Pou4f3 and lacking Prox1. Methods: HCs are killed in the neonatal mouse cochlea using CreER/loxP mouse genetics to drive expression of Diphtheria Toxin, fragment A (DTA) specifically in HCs with the robust ROSA26/CAG-tdTomato reporter line used for fate-mapping. Tamoxifen (3mg/40g) injection given to Atoh1-CreERTM;ROSA26-DTA/CAG-tdTomato mice at P0/P1 produces HC-specific expression of CreER, which results in DTA and tdTomato expression. The CAG promoter in the ROSA26/CAG-tdTomato allele makes its expression much stronger than the ROSA26-DTA allele; thus, while ~99% of HCs are labeled by tdTomato, only ~80% are killed by DTA and the surviving HCs (~20%) express tdTomato. Regenerated HCs do not express tdTomato since they were not present when tamoxifen was injected. Cochlea samples are collected at P4, P7, P15, and P21 and expression of Pou4f3 and Prox1 is assessed by immunostaining and confocal microscopy. Results: In control samples, all HCs express Pou4f3 at all ages tested, but Prox1 expression was only detected in supporting cells. Preliminary experiments demonstrate that in Atoh1-CreERTM;ROSA26-DTA mice at P6, few HCs express Pou4f3 while many HCs express Prox1. However this data is inconclusive since we cannot distinguish between regenerated HCs and surviving HCs. Current experiments are underway to use fate-mapping to label surviving HCs with tdTomato, while regenerated HCs will be tdTomato-negative. We have confirmed that the ROSA26/CAG-tdTomato reporter maintains

expression in ~99% of HCs when two floxed alleles are present using Atoh1-CreERTM;ROSA26-LacZ/CAG-tdTomato mice given tamoxifen at P0/P1.

Disclosures: B.C. Cox: None. **S. Karmarkar:** None.

Poster

722. Mechanoreceptors and Cochlea

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 722.07/GG10

Topic: D.02. Auditory

Title: Effect of stimulus filtering during intense noise exposure on caspase-3 expression and cell death in the auditory hair cells of C57BL/6J mice

Authors: J. RICHARDSON, *J. YODER, B. WILSON, M. ABERNATHY, R. KONIECZKA, A. KOCH, R. TAPP, D. GAUVIN, T. BAIRD;
MPI Res., Mattawan, MI

Abstract: It is well understood that exposure to intense noise causes hearing loss in a variety of animal models. Mice have been shown to be especially sensitive to acoustic overexposure. The specific pathway for auditory hair cell death has also been linked to the frequency and stimulus intensity. The caspase-3 (CASP3) protein is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of programmed cell death. The objective of this experiment was to investigate the potential effects of stimulus filtering on CASP3 mediated apoptotic mechanisms following intense noise exposure. Fifteen female C57Bl/6J mice were exposed to an 8-16 kHz white noise band at 116 dB (filtered for a 55 dB/octave roll-off) for 2 hours. Five animals were then designated for time course analysis of cochlear CASP3 expression and phalloidin-based hair cell analysis at 2, 15, and 30 days following noise exposure. Two days following noise exposure, there was a significant increase in CASP3 activation within the cochlear location tonotopically associated with the noise frequency band. There was severe outer hair cell death observed within the regions directly stimulated by the noise frequency band in addition to a gradient of loss in surrounding regions tonotopically outside of the noise frequency band. Interestingly, these outer hair cells demonstrating a gradient of loss were not expressing CASP3 at this time frame, suggesting a necrotic cell death pathway. By days 15 and 30, the outer hair cell CASP3 expression was decreased in the directly-stimulated cochlear regions and marginally increased in adjacent regions. As expected, the cytocochleograms for these time intervals showed a pattern of

progressive loss as compared to 2 days following exposure. This study corroborates previous reports indicating that lower intensity noise exposure induces auditory hair cell necrosis while higher intensity noise induces auditory hair cell apoptosis. Additionally, the data emphasize the impact of stimulus filtering on the type of auditory hair cell death induced during noise exposure studies.

Disclosures: **J. Richardson:** None. **J. Yoder:** None. **B. Wilson:** None. **M. Abernathy:** None. **R. Konieczka:** None. **A. Koch:** None. **R. Tapp:** None. **D. Gauvin:** None. **T. Baird:** None.

Poster

722. Mechanoreceptors and Cochlea

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Program#/Poster#: 722.08/GG11

Topic: D.02. Auditory

Support: NIH NIA Grant P01 AG009524

Title: Aldosterone neuroprotection via mineralocorticoid receptors in the cochlea of aging CBA/CaJ mice

Authors: X. ZHU[1], B. DING[2], J. P. WALTON[2], *R. D. FRISINA[1];
[1]Chem. & Biomed. Engin. Dept., [2]Communication Sci. & Disorders Dept., Univ. of South Florida, Tampa, FL

Abstract: Spiral ganglion neuron (SGN) degeneration with age is an important biomarker of presbycusis, age-related hearing loss (ARHL). ARHL represents the top communication deficit and neurodegenerative disorder of our aged population. Our previous study indicates a relationship between aldosterone (ALD) serum levels and the severity of ARHL in old human subjects, suggesting that ALD may be involved in the etiology of ARHL (Tadros et al. Hear. Res., 2005). ALD binds with mineralocorticoid receptors (MCR) which we detected at mRNA and protein levels in the young adult CBA/CaJ (CBA) mouse cochlear modiolus (CM). This CM expression suggests that SGNs are possible targets for ALD hormonal influences. To examine this relationship further, a group of middle-aged CBA mice were treated for 4 months with ALD (time-release, subcutaneous pellets), along with an age-matched control group. The ALD treatment group showed higher serum ALD levels compared to young adults and the middle-aged control group, and all three groups were within the normal ALD physiological range for

mice. Auditory brainstem responses (ABRs) were measured longitudinally. The wave I ABR amplitudes were increased in the ALD treated group compared to pretreatment, baseline amplitudes at 12, 24, 32 and 36 kHz, but not in the control group. Positive correlations between SGN cell density and ABRs thresholds shifts were found for the basal turn of the cochlea. The ALD treatment group showed higher MCR protein expression levels in SGNs of the CM. Based on these new findings, it is now apparent that ALD supplementation plays a key role in preserving hearing, reducing SGN degeneration, and stimulating MCR upregulation in the aging cochlea. Supported by NIH Nat. Inst. on Aging Grant P01 AG009524.

Disclosures: X. Zhu: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NIA Grant. **B. Ding:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NIA Grant. **R.D. Frisina:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NIA Grant. **J.P. Walton:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NIA Grant.

Poster

722. Mechanoreceptors and Cochlea

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 722.09/GG12

Topic: D.02. Auditory

Title: Measurments of cochlear microphonics from FM echolocating bats, Japanese house bats, Pipistrellus abramus

Authors: *I. MATSUO[1,2], H. ONODERA[3], H. RIQUIMAROUX[3,2];
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Abstract: The cochlear microhonics (CM) are compound receptor potentials generated primarily by the outer hair cells. The CM, which were changed dependent on frequency and intensity of

sound, reflects the vibration pattern of basilar membrane. It has been shown that CM are recorded outside the cochlea. The CM have been recorded in many kinds of mammals, including several kinds of CF-FM bats. However, CM data from FM bats have not been systematically shown. In this presentation, CMs of FM bats, Pipistrellus abramus, were measured from lower part of brainstem, around the cochlear nucleus, by using tone bursts, frequency-moduated (FM) sounds, and so on as sound stimuli. Characteristics of CM were examined by changing frequency, sound pressure level and spectro-temporal structures of sound stimuli, e. g. FM sounds.

Disclosures: I. Matsuo: None. **H. Onodera:** None. **H. Riquimaroux:** None.

Poster

722. Mechanoreceptors and Cochlea

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Topic: D.02. Auditory

Support: ICMR Grant 45/19/2012-Ana/BMS

AIIMS Grant 218/A-218/2013/RS

Title: Unbiased stereological study of the spiral ganglion neurons in adult human cochlea

Authors: C. KAUR[1], *T. ROY[1], T. NAG[1], A. THAKAR[2], D. BHARDWAJ[3];
[1]Anat., All India Inst. Med. Sci., New Delhi, India; [2]Otorhinolaryngology, [3]Forensic Med. and Toxicology, All India Inst. of Med. Sci., New Delhi, India

Abstract: The auditory pathway is most active and plastic in young individuals. In our study on the cochlear nucleus, we found that compensatory mechanisms arise in the first few decades of life during which humans are involved in productive activity for education or livelihood. We therefore planned to study whether these changes are reflections of alterations occurring at the first order neuron of the auditory system- the spiral ganglion neurons (SGNs). SGNs carry auditory information from the organ of Corti to the cochlear nucleus in the brain stem via the cochlear nerve. There are studies that reveal variable number of neurons in the Spiral ganglia (SG). This may be due to various methodological disparities. Hence, we used unbiased stereological methods to estimate the volume of the SG, the number of neurons found in it and their size. Further, we also wanted to study the commonest inhibitory and excitatory

neurotransmitters involved in the transmission of sound. Hence, we also determined the expression of GABA and NMDA receptors in the SG. Five human temporal bones from adults, who died of various causes, without any known history of inner ear disease or hearing loss, were collected from the mortuary of the All India Institute of Medical Sciences (AIIMS), New Delhi, after obtaining clearance from the institutional ethics committee. These bones were fixed in 4% paraformaldehyde (0.1M PB, pH 7.4), decalcified with ethylenediaminetetraacidic acid (EDTA), cryoprotected and sectioned to obtain 30 µm thick serial coronal sections. Every seventh section was stained with cresyl violet and used for estimating the total volume (Cavalieri probe) of the spiral ganglion, total number of neurons (optical fractionator) and the volume of the soma and its nucleus (nucleator probe) with StereoInvestigator software (Microbrightfield Inc. VT, USA). In addition, some of the sections were used for immunochemical staining for the expression of GABA and NMDA receptors. The mean volume of the SG was 2.14 ± 0.32mm3, the mean number of neurons was 29,411 ± 2074, and the mean volume of the soma and its nucleus were 4482 ± 546µm3 and 308 ± 60µm3 respectively. Immunochemically, we observed that the inhibitory neurotransmitter GABA and the excitatory transmitter glutamate's receptor NMDAR1 were expressed predominantly in the middle and basal turns of the cochlea (responsible for high to mid frequency sounds). This study may provide a new insight in the existing knowledge on morphology of spiral ganglion and provide valuable data in humans for future studies, including quantification of the spiral ganglion neurons in aging and different pathological conditions.

Disclosures: **C. Kaur:** None. **T. Roy:** None. **T. Nag:** None. **A. Thakar:** None. **D. Bhardwaj:** None.

Poster

723. Sound Localization and Binaural Interactions

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NIDCD DC-00436

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Title: Directionality of gecko auditory nerve fibers in free field

Authors: *J. CHRISTENSEN-DALSGAARD[1], C. E. CARR[2];
[1]Univ. Southern Denmark, Odense M, Denmark; [2]Biol., Univ. of Maryland, College Park, MD

Abstract: Lizard ears are ultimate pressure-gradient receivers. Their extreme directionality is created by strong acoustical coupling of the eardrums, with almost perfect transmission from the contralateral ear (Christensen-Dalsgaard and Manley, 2005). Auditory nerve fibers in the Tokay gecko show almost equal sensitivity to ipsi- and contralateral closed-field stimulation, as well as dependence on interaural time and level differences, indicative of strong interaural transmission (Christensen-Dalsgaard, Tang and Carr, 2011). We used laser vibrometry to measure eardrum responses in Tokays, and then exposed the auditory nerve to record single unit responses to free-field sound. The fibers were strongly directional both at low (2-400 Hz) and high frequencies (1-2 kHz) with ovoidal directivities that resembled the eardrum directivity. Geckos are highly vocal, and the nerve fiber directionality to call components was pronounced. Since the auditory nerve fibers showed strong directionality, effectively every neuron in the lizard auditory pathway should be directional, and the processing of sound direction by the CNS may be very different from animals with uncoupled ears. The ovoidal directionality will produce a very strongly lateralized response by simple binaural comparison, which may be sufficient to orient the gecko to sound.

Disclosures: J. Christensen-Dalsgaard: None. **C.E. Carr:** None.

Poster

723. Sound Localization and Binaural Interactions

Location: Halls A-C

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Topic: D.02. Auditory

Support: NIH grant DC007690

Title: Heterogeneous organization of neuronal tunings for spatial cues in the primary avian auditory field: Comparison of the topographic midbrain with the non-topographic forebrain

Authors: *M. V. BECKERT, J. L. PENA;
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: We are investigating the organization of neuronal tunings to binaural cues important in localizing sound in the primary auditory forebrain structure (Field L) of the barn owl, Tyto alba. Previous work has suggested forebrain structures like Field L are organized into clusters of similarly tuned neurons. We improved upon previously used techniques by utilizing tetrode recordings to directly address the existence of these clusters. Tetrode recordings were performed in anesthetized barn owls. Dichotic sound stimuli were presented through custom made earphones. The interaural time (ITD) and level difference (ILD) of broadband or pure tune stimuli were varied to obtain tuning profiles of multiple single units within a single recording site. Tuning profiles of units within these local populations were then compared to one another to assess the similarity or difference in their composition. Our results are inconsistent with the clusters hypothesis and suggest a more complex and heterogeneous organization of neuronal tunings similar to the mammalian primary auditory cortex (A1). Groups of neighboring neurons can be tuned to very different combinations of binaural cues. This is observed by assessing the variety of preferred ITD and ILD within a single site as well as the lack of correlation of each unit's tuning profiles compared to one another. This heterogeneous organizational motif stands in stark contrast to the auditory midbrain of the barn owl which contains a topographic map of auditory space. Here we find neighboring neurons are similarly tuned to one another with very small differences in their preferred tunings as well as having their tuning profiles correlated to one another. Our findings suggest the avian auditory forebrain is more complexly organized and may be more similar to mammalian A1 than first thought. Further work must be done to determine the utility of a heterogeneous tuning organization on neural representation.

Disclosures: **M.V. Beckert:** None. **J.L. Pena:** None.

Poster

723. Sound Localization and Binaural Interactions

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Topic: D.02. Auditory

Support: BBSRC (BB/J008680/1)

Title: Commissural gain control improves discrimination of sound localization cues in the auditory midbrain

Authors: *L. ORTON, C. PAPASAVVAS, A. REES;
Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: The accurate localization of sound sources is essential for animal survival. The inferior colliculi (ICs) are the first point in the mammalian auditory pathway where the cues used to localize sounds in the horizontal plane, interaural time discrepancies (ITDs) and interaural level discrepancies (ILDs), are both represented. These cues are first analyzed independently on each side of the midline, in the medial and lateral superior olive of the brainstem, before projecting up the auditory pathway to each IC. Due to this segregation, each IC predominantly represents stimuli in the contralateral hemifield. We hypothesized that athwart connections between the ICs via the commissure of the inferior colliculi may allow each IC to exert reciprocal gain control over the other to improve the representation of these cues. To test this hypothesis we recorded extracellular responses from 51 IC neurons sensitive to ITDs or ILDs in 20 urethane anaesthetized adult guinea pigs, before, during, and following recovery from deactivation of the contralateral IC by cryoloop cooling or microdialysis of procaine. Contralateral deactivation could increase or decrease the firing rate of ITD or ILD firing rate response functions. Changes in firing rate were described by linear gain functions. The majority of ITD and ILD response functions were rescaled by divisive and additive gain. These changes caused a reversible decrease in the dynamic ranges of ITD and ILD response functions which reduced the ability of changes in firing rate along the ITD or ILD axes to signal changes in sound location - as determined by ROC analyses. These data show for the first time that each IC exerts reciprocal multiplicative and subtractive gain control over the other IC. This gain control operates similarly on both ITD and ILD sensitive neurons, and enhances the ability of IC neurons to signal changes in azimuthal sound source location. This mechanism may be required to extract a coherent representation of ITDs and ILDs in the IC from the convergence of the numerous, diverse afferent sources each IC receives and integrates.

Disclosures: **L. Orton:** None. **C. Papasavvas:** None. **A. Rees:** None.

Poster

723. Sound Localization and Binaural Interactions

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Topic: D.02. Auditory

Support: NIH/NIDCD grant DC008989

Title: Sound localization processing nuclei of the gerbil contain functional α7 nAChRs

Authors: *S. R. WEIMANN, E. C. ESPOSITO, R. BURGER;
Biol. Sci., Lehigh Univ., Bethlehem, PA

Abstract: The α7 nicotinic acetylcholine receptor (nAChR) has broad distribution throughout the mammalian brain (Dominguez et al. 1942; Marks et al. 1996; Morley et al. 1977). The functional properties of this receptor can vary based on it cellular location and developmental expression. Presynaptic expression of α7 nAChRs is known to facilitate neurotransmitter release due to it's high calcium permeability (Albuquerque et al. 1997; McGehee et al 1995) while postsynaptic expression can have broad effects including the regulation of gene expression, induction of process remodeling, neuron survival and the mediation of excitatory currents. Happe and Morley (2004) showed that, the α7 nAChR subunit is richly expressed in the superior olivary complex (SOC). However, expression and physiological relevance amongst SOC nuclei involved in the computation of sound location had yet to be determined. We examined the expression of the α7 nAChR within the SOC using immunohistochemistry (IHC). IHC was performed on paraformaldehyde (PFA) fixed tissue from gerbils aged p19-p60 (α7 nAChR: mouse monoclonal; 1:400; Sigma; cat. # M220; lynx1: rabbit polyclonal; 1:1000). The tissue was counterstained with MAP2 (chicken;1:1000; Neuromics; cat. # CH22103). Tissue was examined using confocal microscopy. We demonstrate that the α7 nAChR is expressed in the medial superior olive (MSO), the lateral superior olive (LSO), and the medial nucleus of the trapezoid body (MNTB). The functionality of α7 nAChRs was examined using *in vitro* whole-cell patch clamp on brain stem slices obtained from gerbils age P9-14. Carbachol, a cholinergic agonist, was applied using a 250 mS puff while other drugs were bath applied. Puff application of carbachol evoked inward currents that were blocked by the α7 subunit specific antagonist methyllycaconitine (MLA). These results demonstrate the presence of functional α7 nAChRs on postsynaptic sites within SOC nuclei and that cholinergic signaling via α7 nAChRs does have physiological relevance in the mammalian sound localization pathway. This suggests that cholinergic signaling may contribute to the development of the sound localization circuitry or mediate excitatory currents involved in the precision of temporal coding.

Disclosures: S.R. Weimann: None. **E.C. Esposito:** None. **R. Burger:** None.

Poster

723. Sound Localization and Binaural Interactions

Location: Halls A-C

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Program#/Poster#: 723.05/GG18

Topic: D.02. Auditory

Support: DC006788

Title: Evidence for a distance-dependent gradient in the strength of excitatory synapses along the dendrites of coincidence detector neurons in the medial superior olive

Authors: B. D. WINTERS[1], S.-X. JIN[1], *N. L. GOLDING[2];
[1]Dept. of Neurosci., [2]Univ. of Texas At Austin, AUSTIN, TX

Abstract: The neurons of the medial superior olive (MSO) receive excitatory input derived from both ears and detect sub-millisecond differences in timing that are used to localize sounds along the azimuth. The biophysical properties of MSO dendrites result in strong amplitude attenuation of excitatory postsynaptic potentials (EPSPs) as they propagate from the distal dendrites toward the soma and axon. We hypothesized that the impact of distal inputs would be diminished unless they were stronger than proximal inputs. To test this we performed dual whole cell recordings to measure miniature EPSPs (mEPSPs) simultaneously at the soma and in the dendrite using gerbil brainstem slices (P16-25). In the presence of 1 μM strychnine to block glycinergic inhibition, we detected mEPSPs using a dual-channel template matching algorithm and averaged only those events initiated nearest the dendritic recording location (mEPSPs exhibiting the highest 10% attenuation between recordings sites). Miniature EPSPs propagating from the dendrites to the soma showed strong attenuation (up to 13-fold), reflecting the low input resistances (<10 MΩ) found in the soma and dendrites of these cells. EPSP attenuation vs. propagation distance was described by a single exponential function (space constant $\lambda = 48$ μm). The absolute amplitude of the local dendritic mEPSPs increased nearly 6-fold over the first 90 μm of the dendrite (0.2 to 1.2 mV from 24 to 90 μm), while the amplitude of these events after propagation to the soma remained uniform, at ~0.2 mV. In a separate series of experiments, we bath-applied 0.2 mM calcium to globally reduce synaptic release probability, and then locally restored normal release probability at dendritic locations of interest with focal application of 4 mM calcium. Putative unitary EPSPs at these sites were induced with synaptic stimulation only at the selected locations and recorded at the soma. We probed locations along the dendrites in this way, ranging from 33 to 123 μm from the somatic recording electrode. Comparable to the mEPSP analyses, we found that the amplitude of EPSPs recorded at the soma were largely independent of the distance along the dendrite (avg. 0.56 mV). Together these data strongly suggest that the strength of excitatory inputs onto MSO neuron dendrites increase with distance from the soma, counterbalancing the effects of the passive and active cable properties that attenuate as EPSPs as they propagate toward the soma from the dendrites.

Disclosures: B.D. Winters: None. **S. Jin:** None. **N.L. Golding:** None.

Poster

723. Sound Localization and Binaural Interactions

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Champalimaud Foundation

Title: Level invariant inter-aural level difference discrimination in rats

Authors: *J. L. PARDO-VAZQUEZ, A. RENART;
Champalimaud Ctr. For the Unknown, Lisboa, Portugal

Abstract: The relationship between neural variability and the accuracy of perception can be addressed by recording neuronal populations during a perceptual decision-making task. This strategy imposes two constrains on the task: First, the physical features of the stimulation delivered to the brain have to be strictly controlled. Second, the impact of non-sensory factors has to be minimized to ensure that behavioral choices are a reliable proxy for what the animal perceives. As a first step in this direction, we have developed a two-alternative forced-choice auditory discrimination task aimed at satisfying both requirements. In the task rats have to discriminate the difference in level between two white-noise bursts presented simultaneously to each ear (ILD) through custom-made detachable ultrasonic headphones. In order to earn water reward, rats have to make a response from a central poke towards a lateral poke to the side where the presented stimulus is louder. Because ILD is the binaural cue used by rats to localize sound in the horizontal plane (Wesolek et al., Hear. Res., 2010), the task capitalizes on the natural tendency of rats to orient to the location of a source. This, and the fact that the threshold for detection of lateralization is hard wired, facilitates training and makes the task less susceptible to non-sensory factors like history effects. Consistent with the ethologically natural set of contingencies imposed by the task, rats learn to perform ILD discriminations with psychophysical thresholds of 1-1.5 dB SPL within 4-5 sessions lasting 1000-1500 trials each. In order to confirm that the rats are indeed using ILDs for performing the task, we presented stimuli with a fixed set of ILDs but different average binaural levels (ABL) of 20, 40 and 60 dB SPL, in blocks of 80 trials. Psychometric curves at ABLs of 40 and 60 dB SPL are not different, while ILD discriminability at an ABL of 20 dB SPL drops. However, performance in a task requiring only detection of a single burst presented to one ear was more than 95% accurate at 40 and 60 dB SPL, but accuracy was significantly lower for 20 dB SPL. We thus conclude that rats can and are indeed performing (level invariant) ILD discriminations in our task when stimuli are above their detection threshold. Discrimination difficulty affects both accuracy and reaction time, with

significant differences between easy and difficult decisions in both performance measures. This suggests that, at least to some degree, rats accumulate sensory evidence during the stimulus presentation. We are currently extending the task to include time-varying ILDs, which will allow us to explore how the impact of the stimulus on choice varies across time.

Disclosures: J.L. Pardo-Vazquez: None. **A. Renart:** None.

Poster

723. Sound Localization and Binaural Interactions

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Title: Effects of interaural decorrelation on neural and behavioral sensitivity to interaural level differences

Authors: A. D. BROWN, *D. J. TOLLIN;
Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Interaural differences in signal timing and level (ITDs and ILDs) associated with the angular displacement of a sound source relative to the head provide the major cues to source location in mammals. In many listening environments, temporal and intensitive distortions of the binaural signal associated with reflections and reverberation act to degrade ITDs and ILDs, reducing behavioral and neural sensitivity to sound source location. Similar degradations are thought to be experienced by hearing-impaired humans who use hearing aids or cochlear implants, as these devices degrade temporal and intensitive features of the signal at each ear. Evidence suggests that ILD sensitivity is relatively robust to *temporal* distortions of the binaural signal, i.e. interaural decorrelation. However, because neural coding of ILD depends on antagonistic summation of excitatory and inhibitory inputs from each ear within a relatively brief (≤1-2 ms) window of integration, interaural decorrelation of sufficiently low-frequency signals or of low-frequency amplitude-modulation envelopes should theoretically lead to reduced ILD sensitivity. To test this hypothesis we used synthetic stimuli, which included noise bursts and filtered impulses of varied interaural correlation. Behavioral data were obtained from human listeners using a psychophysical paradigm that simultaneously assessed the detectability and salience of imposed ILDs. Neural data were obtained extracellularly from single neurons in the

inferior colliculus of anesthetized chinchillas, a species that is audiometrically comparable to humans. Preliminary data suggest that interaural decorrelation of sufficiently low-frequency signals, or decorrelation of low-frequency amplitude-modulated envelopes of high-frequency signals, reduce but do not eliminate behavioral and neural ILD sensitivity. Data are discussed in the context of a physiologically-inspired model of ILD coding, with relevant comparisons to ITD coding.

Disclosures: A.D. Brown: None. **D.J. Tollin:** None.

Poster

723. Sound Localization and Binaural Interactions

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 723.08/GG21

Topic: D.02. Auditory

Support: Wellcome Trust Principal Research Fellowship WT076508AIA

Newton Abraham Studentship, University of Oxford

Early Career Research Fellowship, University of Oxford

Title: Fine spatial representation of interaural level differences in the auditory cortex: a two-photon imaging study

Authors: M. PANNIELLO, A. J. KING, J. C. DAHMEN, *K. M. WALKER;
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Abstract: Hearing research has been revolutionized by the recent application of *in vivo* two-photon calcium imaging to the study of auditory cortex, thanks to the high spatial sampling rate of this technique. The topographic representation of sound frequency, recognized as the main organizational principle of primary auditory cortex, has been shown to be absent at a fine spatial scale (within tens of micrometers). Here, we use two-photon calcium imaging to investigate the responses of neurons in auditory cortex to sound level differences between the two ears (Interaural Level Differences; ILD). Some previous extracellular recording studies have found evidence of a topographic organization of binaural response properties across the auditory cortical surface, but ILD preferences have usually been shown to lack a cortical organization. Stereotaxic injections of an AAV vector carrying GCaMP6m, a genetically encoded calcium indicator, were performed in the auditory cortex of mice aged 6-7 weeks. Two-photon imaging

of auditory cortical activity was carried out in the anesthetized mouse 3-6 weeks after this injection. During imaging, we presented 80dB SPL noise bursts with 0-30dB ILD between the two ears, as well as monaurally to each ear. We found a patchy arrangement of binaural preference. Small neuronal clusters (50-60um²) showing a common preferred ILD were often adjacent to clusters with a different (even contralateral) ILD tuning. We did not, however, find evidence for binaural bands in the mouse auditory cortex. These results are in accordance with previous electrophysiology studies that propose a poorly ordered spatial representation of ILD in the auditory cortex at a large spatial scale. However, the dense spatial sampling of 2-photon imaging led to the novel discovery of locally clustered ILD preferences among neighboring auditory cortical neurons.

Disclosures: M. Panniello: None. **A.J. King:** None. **J.C. Dahmen:** None. **K.M. Walker:** None.

Poster

723. Sound Localization and Binaural Interactions

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Topic: D.02. Auditory

Support: Alexander von Humboldt Foundation

DFG (SFB 870/2_A10; KO 2207/3-1)

Technical Support by Mrs. Hilde Wohlfrom

Title: The mouse medial superior olive

Authors: R. M. BURGER[1], M. J. FISCHL[2], O. ALEXANDROVA[2], I. D. FORSYTHE[3], *C. KOPP-SCHEINPFLUG[2];
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Abstract: Dedicated binaural neural pathways process sound location information, which is computed largely by comparison of sound input between the two ears. For example, low frequency sound localization is dependent on arrival time differences of sound at the two ears, or interaural time disparities (ITDs). In mammals, ITDs are processed in the medial superior olive,

where excitatory and inhibitory inputs evoked by each ear impart spatial selectivity in MSO neurons. However, for most small mammals including mice, small inter-ear distances and poor low-frequency hearing means that physiologically relevant ITDs are very limited. It has been postulated that the MSO is therefore unnecessary in mice, and indeed it has rarely been investigated or described. While the MSO has been a focus of intense study in several animals over the last few decades, the "missing mouse MSO" problem has inhibited application of genetic tools that proved invaluable in other auditory brain regions. Here we report a population of neurons in the mouse superior olivary complex that are strong candidates for an MSO based on their: bipolar shape, ventral location between MNTB and LSO, single action potential response to sustained current injection, large and fast IH currents and prominent KV1.1 currents. These putative mouse MSO neurons also receive bilateral excitatory input and contralateral, MNTB-mediated inhibitory input. Additionally, their major output projects to the ipsilateral inferior colliculus. Finally, we show that these MSO neurons are also subject to modulation by nitric oxide, similar to their neighbours in the superior paraolivary nucleus and the medial nucleus of the trapezoid body. The presence of MSO cells in mice that physiologically and anatomically resemble those of cat, rat and gerbil, will provide access to an animal model in which genetic manipulation can be used to address some additional questions about the ITD circuit.

Disclosures: R.M. Burger: None. **M.J. Fischl:** None. **O. Alexandrova:** None. **I.D. Forsythe:** None. **C. Kopp-Scheinpflug:** None.

Poster

723. Sound Localization and Binaural Interactions

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supported by“the Fundamental Research Funds for the Central Universities”

Title: Effects of interaural delay on human cortical processing of changes in interaural correlation

Authors: L. KONG[1], R. NING[2], X. ZHANG[2], *M. WANG[2,3];
[1]Dept. of physiology and pharmacology, Univ. of Calgary, Calgary, AB, Canada; [2]Psychology, Sch. of Psychology, Beijing, China; [3]Beijing Key Lab. of Applied Exptl. Psychology, Beijing, China

Abstract: Human listeners are sensitive to a change in interaural correlation (IAC, from 0 to 1, or from 1 to 0), even when an interaural delay (larger than the physiological interaural time difference) is introduced. Cortical responses to a bidirectional IAC (from 0 to 1, then back to 1) have been proved to be affected by interaural delay. However, it remains unclear whether the interaural delay modulates the cortical responses to unidirectional IAC (from 1 to 0 vs from 0 to 1) in a different manner. The current study investigated effect of interaural delay on the event-related potential responses to IAC in different directions. We found that both the upward IAC (from 0 to 1) and the downward IAC (from 1 to 0) evoked obvious cortical responses. These cortical responses were significantly decreased as the interaural delay increased from 0 to 4 ms. In addition, the cortical responses to upward IAC were more susceptible to the interaural delay than those to the downward IAC, and the decrease of cortical responses to upward IAC was significantly faster than that of responses to downward IAC. Thus, our data suggest that the interaural delay affects cortical processing of upward and downward IAC in a different way. This direction-specific modulation appears to be expected if temporal jitter (loss of neural synchrony in the auditory system) increases with interaural delay.

Disclosures: L. Kong: None. **R. Ning:** None. **X. Zhang:** None. **M. Wang:** None.

Poster

723. Sound Localization and Binaural Interactions

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Topic: D.02. Auditory

Support: NIH R01-DC011548

Title: Task-related attention affects the cortical processing of auditory spatial cues

Authors: *N. C. HIGGINS, G. C. STECKER;
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Abstract: Spatial processing in the auditory cortex depends on internal calculations of interaural level (ILD) and time differences (ITD) at the two ears. In individual cortical neurons, changes of firing rate as a function of ILD or ITD demonstrate sensitivity to these cues, with generally greater responses to contralateral than to ipsilateral stimuli, a response profile also observed in human fMRI studies. Engagement in specific tasks can modulate this sensitivity by sharpening single unit responses during localization tasks as compared to passive listening. While human fMRI studies have shown increased cortical activation in auditory cortex during tasks that require attention and memory, no studies to date have examined the role of task engagement during processing of auditory spatial cues in humans. To examine this question, continuous event-related fMRI was used to measure cortical responses to amplitude-modulated noises varying parametrically in ILD or ITD. Task type (visual, auditory location, or auditory pitch) varied between epochs of 10 trials, each of which potentially carried a target of any type: location (change in ILD or ITD), pitch (change in burst rate), or visual (change in brightness). Subjects indicated the direction of each target when cued. Following preprocessing, functional data were subjected to a standardized hemodynamic regression analysis, and projected to the cortical surface using Freesurfer. Robust responses to auditory stimuli were observed in voxels primarily clustered around Heschl's Gyrus (HG) and posterior superior temporal gyrus (STG). Significant main effects of cue (ILD and ITD) were observed in both these regions, with the highest activation in response to contralateral stimuli (tested with repeated measures ANOVA). Engagement in auditory tasks (detection of location or pitch targets), further enhanced contralateral responses compared to detection of visual targets, and significant effects were thus observed in a larger range of cue conditions. This pattern of increased activation was mostly observed in posterior STG, outside conventionally defined HG. Activity in HG generally reflected cue sensitivity regardless of task condition, whereas posterior STG was most active when engaged in auditory-specific tasks. This pattern of results supports a processing model in which HG processes initial lateralized spatial information, while posterior STG is more dependent on task related attention, potentially reflecting extraction of higher level sound features.

Disclosures: **N.C. Higgins:** None. **G.C. Stecker:** None.

Poster

723. Sound Localization and Binaural Interactions

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Support: NIH-NIDCD R00-010206

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NIH-NIDCD T32-DC-00046

Title: Characterization of interaural differences in electrical firing patterns of unsynchronized bilateral cochlear-implant processors

Authors: *F. A. RODRIGUEZ CAMPOS, M. J. GOUPELL;
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Abstract: Bilateral cochlear-implant (CI) speech processors are not currently binaurally-synchronized, which could introduce spatial distortions. These potential distortions are commonly assumed to be a major contributor to the poor spatial hearing abilities of bilateral CI users. Since the magnitude of the spatial distortions have yet to be empirically measured in unsynchronized CI speech processors, we measured the changes to static and time-varying interaural time and level differences that were caused by these devices. Behind-the-ear Cochlear Ltd. speech processors were set to either a 12-channel constant stimulation (i.e., continuous-interleaved simulation, CIS) strategy or a 22-channel peak-picking (i.e., advanced combination encoding or ACE) strategy. Both strategies were set to stimulate at 900 pulses/s per electrode. The processors had a dynamic range of 40 dB, the automatic gain control (AGC) was enabled, and the adaptive dynamic range optimization (ADRO) was disabled. Processors were set for everyday use to minimize effects of microphone beam focusing schemes. Each processor was connected to an implant in a box and the electrical pulse train outputs were measured from single electrodes. The CI processors were placed on the ears of an acoustic mannequin (KEMAR) in an anechoic chamber. Using a speaker array, sound sources were presented at 65 dB-A from different locations in the horizontal plane +90 to -90 degrees in 11.25 degree steps. The stimuli consisted of pure tones, sinusoidal amplitude modulated (SAM) tones, Gaussian pink noise, SAM noise, words, and sentences. The interaural time and level differences for the different stimuli were compared between the acoustic stimuli measured with the KEMAR microphones and the electrical stimuli recorded from the speech processors. The results will inform us on the necessity for using binaurally-synchronized CI speech processors.

Disclosures: F.A. Rodriguez Campos: None. **M.J. Goupell:** None.

Poster

723. Sound Localization and Binaural Interactions

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Topic: D.02. Auditory

Support: NIH F32 DC012978

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Title: Ephaptic effects alter spiking in a model of coincidence detector neurons in auditory brainstem

Authors: *J. H. GOLDWYN[1,2], J. RINZEL[1,2];
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Abstract: The activity of neurons in the brain creates a "bath" of extracellular voltage (V_{ext}). The functional consequences, if any, of endogenous V_{ext} remain largely unexplored. We are investigating whether V_{ext} in the auditory brainstem influences temporally precise computations in the medial superior olive (MSO). V_{ext} in the MSO is distinct from cortical local field potentials: it is large (mV scale) and exhibits fast (kHz scale), sound-evoked oscillations. Post-synaptic membrane currents in MSO neurons are thought to generate this prominent V_{ext} (Mc Laughlin et al. 2010, e.g.) with negligible contributions from back-propagating action potentials. MSO neurons have no direct synaptic connections, so *ephaptic* interactions (through V_{ext}) would be a novel mechanism for coupling MSO neurons. MSO spiking is sensitive to submillisecond timing differences in their synaptic inputs (Yin and Chan 1990) and i*n vitro* experiments have demonstrated that weak V_{ext} can alter spike timing (Anastassiou et al., 2011). These observations suggest that ephaptic effects may influence the capacity of the MSO to encode temporal information. We tested ephaptic coupling with a biophysically-based MSO model (Mathews et al. 2010) that we dynamically couple to V_{ext}. We simulated the V_{ext} produced by MSO neurons and then compared spiking activity in the presence or absence of V_{ext}. The simulated V_{ext} reproduces important features of V_{ext} recorded *in vivo* in response to acoustic stimuli such as a dipole-like spatial profile (monaural responses) and large-amplitude oscillations entrained to the frequency of tone stimuli (Goldwyn et al. 2012). We find that V_{ext} on the order ~1 mV can perturb membrane potential of a "resting" neuron by ~1 mV. These modest changes in membrane potential can alter spike initiation and timing in response to random synaptic inputs (homogeneous Poisson process inputs) and inputs temporal structure (interaural time differences). In our simulations, the "direction" of ephaptic coupling depends on the location of the spike generating axon initial segment (AxIS). For V_{ext} generated by coincident bilateral inputs, ephaptic coupling is hyperpolarizing/inhibitory if the AxIS is proximal to the soma of the MSO (spike thresholds increase and firing rates decrease in the presence of a background V_{ext}). It is depolarizing/excitatory if the AxIS is distant from the soma. We conclude that endogenous V_{ext}

may alter spiking dynamics in neural populations that perform temporally precise computations. Our work highlights how ephaptic effects can depend sensitively on cellular morphology and the anatomy of spike generators.

Disclosures: J.H. Goldwyn: None. **J. Rinzel:** None.

Poster

723. Sound Localization and Binaural Interactions

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Topic: D.02. Auditory

Support: French FRM

European Research Council (Stg 240132)

Title: A model of sound source localization based on frequency dependent ITD processing

Authors: *P. YGER, V. BENICHOUX, R. BRETTE;
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Abstract: To localize sounds in the environment, animals mostly use spectro-temporal cues originating from the physical disparities of the sound waveforms impacting the ears. Among those, the Interaural Time Difference (ITD) has been shown to be crucial in mammals for locating low-frequency sounds, and is known to be processed by neurons in a particular structure, the Medial Superior Olive (MSO). While it is classically considered that the emergence of ITD selectivity in a neuron of the MSO is due to differences in the axonal delays originating from the two ears and impinging the cell (the so called “delay-line” or Jeffress model), experimental evidence shows that the best delay (the ITD at which the neuron’s firing rate is maximum) is also dependent on the frequency of the sound. Recently it has been suggested that such frequency-dependent best delay tuning reflects the frequency-dependent ITDs found in ecological environments. A proposed mechanism is that cells receive inputs not only with different axonal delays from both ears, but also from different frequency bands. Since it challenges the classical model, we investigated the emergence of binaural tuning in the MSO through Hebbian learning, similarly to what has already been done in [1]. We built a realistic neuronal architecture based on spiking neurons and using homeostatic and spike-timing dependent plasticity rules for synapses from cochlear nucleus projections to neurons in the MSO.

By training the system with binaural sounds, we were able to study the development of ITD selectivity for various inputs and to understand why, and how, this ITD selectivity can depend on the frequency of the sound. Finally, we will discuss, from a coding point of view, the potential implications raised by the frequency dependence of the best delay. As pointed out by recent work [2], with such a frequency-dependent best delay, neurons in the MSO should be seen as coding for complex features in the interaural phase spectrum of natural sounds, rather than for just a fixed delay difference. Acknowledgments This work is funded with a Fellowship Grant from the French FRM (Foundation for Medical Research), and the European Research Council (StG 240132)/ References 1. Fontaine B and Brette R, Neural Development of Binaural Tuning through Hebbian Learning Predicts Frequency-Dependent Best Delays, J. Neurosci. 2011, 31(32):11692-11696 2. Benichoux V, Fontaine B, Karino S, Joris P and Brette R, Frequency-dependent time differences between the ears are matched in neural tuning (submitted)

Disclosures: P. Yger: None. **V. Benichoux:** None. **R. Brette:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: D.03. Multisensory

Support: FOMIX CONACYT-GOBIERNO DEL ESTADO DE CAMPECHE Núm. 0170573

UNIVERSIDAD AUTONOMA DE CAMPECHE

Title: Visual, auditory and somatosensory long-latency evoked potentials in patients with diabetes mellitus 2

Authors: *O. H. HERNANDEZ[1,2], R. GARCIA-MARTINEZ[2], L. AGUIRRE-MANZO[1], F. YE-EHUAN[1], G. MALDONADO-VELAZQUEZ[2], C. G. KU-MENDEZ[2];
[1]HOSPITAL GENERAL DE ESPECIALIDADES DR. JAVIER BUENFIL OSORIO, CAMPECHE, Mexico; [2]Ctr. de investigaciones biomedicas, UNIVERSIDAD AUTONOMA DE CAMPECHE, Campeche, Mexico

Abstract: There is a lot of information on the effects of diabetes mellitus type 2 (DM2) in peripheral neurons, but much less in central neurons. A better understanding of the effects of this disease in the sensory systems will open more opportunities to prevent its complications. The P2

is a robust wave that appears in the averaged recordings of the long-latency evoked potentials (LLEP). P2 is endogenous, represents the process of stimulus identification, and is generated in the frontal associative cortex. Our lab has recently shown that the P2 parameters are dependent on the sensory system used. In healthy subjects, the auditory stimulation causes faster and larger brain responses than the visual stimulation, while the somatosensory is intermediate (Hernández et al., 2014 Clin EEG Neurosc In press). Although considerable progress has been made in figuring out the underlying mechanisms of P2 generation, we emphasize the need to understand how the DM2 affect the LLEPs in a multisensory design before attempting to determine its underlying cellular machinery. It is already known that the brain responses are longer and smaller in patients with DM2 vs controls, but three parameters (rate of rise, amplitude and peak latency) of the P2 component of the LLEP have never been analyzed together in a multimodal paradigm in DM2 patients. Then the goals of this research were measure and compare these electrophysiological parameters in diabetic patients applying trains of visual, auditory and somatosensory stimuli. The task consisted of 64 trials that present repeated sensory stimuli at 1 Hz using the VikingQuest SystemTM. Auditory, visual and somatosensory stimuli were administered to each patient while EEG recordings were taken at Cz. This experiment tested 11 patients with DM2 (3 female) with a mean (SD) age of 49.7 (10) yrs; 32.4 (6.0) BMI; 9.4 (4.5) years with the disease; 126.9 (45.7) mg/dL of blood glucose; and 7.69 (1.0) % of HbA1c. The data were analyzed by 3 (stimuli) ANOVA and pair comparisons with Bonferroni test. The results showed clear sensory-dependent differences in peak latency [$F(2,48)=23.6$, $p<.0001$], amplitude [$F(2,34)=8.25$, $p<.001$] and rate of rise [$F(2,46)=3.99$, $p<.025$] parameters, where the somatosensory stimuli produced the slowest and smallest brain waves. A negative correlation was obtained between blood glucose and P2 amplitude in the somatosensory system ($r=-0.698$; $p<.036$) but not in the visual or auditory modalities. These results provide support to a sensory-dependent damage of the DM2 and suggest that this disease produces stronger effects in the somatosensory pathway than in the visual or auditory systems.

Disclosures: O.H. Hernandez: None. **R. Garcia-martinez:** None. **L. Aguirre-manzo:** None. **F. Ye-ehuan:** None. **G. Maldonado-velazquez:** None. **C.G. Ku-mendez:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: D.03. Multisensory

Support: NWO Grant: VICI

Title: Visuo-tactile interactions are dependent on the predictive value of the visual stimulus

Authors: *M. KANDULA, D. HOFMAN, C. DIJKERMAN;
Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands

Abstract: In this study we aimed to explore the predictive link between visual stimuli moving towards the body and the tactile consequences that follow. More specifically, we tested if information derived from an approaching visual stimulus, could be used to make more accurate judgments about the location and time of the impending tactile contact associated with that stimulus. In order to do this, we used moving arm stimuli, displayed on a computer screen, which appeared to travel either towards the face (middle of the left/right cheek) or slightly away from the subject's face followed by tactile stimulation on the left/right cheek. The time lag between the visual stimulus and tactile stimulation was also manipulated to simulate tactile contact at a time that was either consistent or inconsistent with the speed of the approaching hand. Reaction time information indicated that faster responses were produced when the arm moved towards the hemispace in which the tactile stimulation was delivered and was insensitive to whether the arm was moving towards the cheek or slightly away from the cheek. Also, the response time was fastest when the tactile stimulation arrived at the moment that was consistent with the speed of the moving arm. These results suggest the existence of a predictive mechanism that exploits the visual information derived from objects moving towards the body for making judgments about the time and location of impending tactile contact.

Disclosures: M. Kandula: None. **D. Hofman:** None. **C. Dijkerman:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

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Topic: D.03. Multisensory

Support: Swedish Research Council - Post doc grant

Title: Dual site TMS as a tool to investigate fronto-parietal connectivity during the rubber hand Illusion

Authors: *A. KARABANOV[1], A. RITTERBAND-ROSENBAUM[3], M. SCHRAM CHRISTENSEN[3,4], H. SIEBNER[2], J. NIELSEN[3];
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Abstract: Introduction: In recent years a growing number of studies have used the Rubber Hand Illusion (RHI) (Botvinick and Cohen, 1998) to experimentally manipulate body ownership and investigate the neural underpinnings of self-awareness. These studies have concordantly implicated the importance of the anterior intraparietal sulcus (aIPS) in adjusting body ownership during the RHI. In this study we use dual-site transcranial magnetic stimulation (Karabanov, 2013) (dsTMS) to investigate parietal-motor connectivity during the RHI. Methods: Motor evoked potentials (MEPs) were recorded from the first dorsal interossous (FDI). MEPs were sampled at rest (baseline) and during a motor RHI paradigm (Kalckert, 2012). The participant's hand was hidden but mechanically connected to a visible rubber hand (RH). Three conditions were tested: a) RH placed in an anatomically plausible position, inducing agency and ownership, b) RH placed in an anatomically implausible position, inducing only agency and c) mechanical connection disrupted inducing neither agency nor ownership. To quantify illusion strength participants completed a questionnaire and a perceptual pointing task after every condition. In a first experiment (n=8) single TMS pulses where given to primary motor cortex (M1) to test if an illusion could be induced during TMS. In a second experiment (n=18) dsTMS was applied. The test pulse (TP, adjusted to 1mV) was given alone or preceded by a conditioning pulse (CP, 90%rMT) over the aIPS at inter-stimulus intervals (ISIs) of 2,4,6,8ms. Preliminary Results: Experiment 1: Self-report and perceptual drift indicated that only condition a) induced a RHI. A rmANOVA on the MEP size did not show significant differences between conditions a, b and c. Experiment 2: Self-report and perceptual drift indicated that only condition a) induced a RHI. The inhibitory effect of a CP on M1 at rest was confirmed. AN rmANOVA of the MEP size showed show a main effect of Condition ($p = 0.006$). Post-hoc tests showed that conditions b) and c) (agency and control) where significantly higher than baseline ($p = 0.005$ and 0.04 respectively). Discussion: Our results show that it is possible to induce a RHI during TMS and that the RHI does not alter M1 excitability (Experiment 1). Our dsTMS data shows that movement modulates aIPS-M1 connectivity. This modulation is stronger in conditions not causing a RHI. However, since condition a) was not significantly different from either baseline or control it is uncertain if this constitutes an ownership specific effect. The general effect of movement seems to disrupt the parietal inhibition observed at rest and interfere with subtler ownership modulations.

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Poster

724. Multisensory: Cross-Modal Processing in Humans

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Topic: D.03. Multisensory

Support: CIHR fellowship

Title: Modulation of sensorimotor integration by rubber hand illusion assessed with transcranial magnetic stimulation

Authors: *R. ISAYAMA[1,2], G. JEGATHEESWARAN[1,2], M. VESIA[1], B. ELAHI[3], C. GUNRAJ[1], L. CARDINALI[4], A. FARNE[5], R. CHEN[1,2];
[1]Div. of Brain, Imaging and Behavior - Systems Neurosci., Toronto Western Res. Inst., Toronto, ON, Canada; [2]Med., [3]Neurol., Univ. of Toronto, Toronto, ON, Canada; [4]The Brain and Mind Inst., Univ. of Western Ontario, London, ON, Canada; [5]Lyon Neurosci. Res. Ctr., Lyon, France

Abstract: <Background> Sensory inputs of different modalities need to be properly integrated into a coherent representation in the brain. This multi-modal sensory processing is critical for sensorimotor integration, which combines sensory inputs with motor plan or outputs to generate accurate movements. The physiology of multi-sensory integration is not well understood because of difficulties in examining the multi-sensory integration separate from motor system. Therefore, we used the rubber hand illusion (RHI) paradigm, which evokes a multisensory integration of conflicting visual and tactile inputs from a rubber hand and subject's own hand resulting in an illusory perception of ownership over the rubber hand and a shift of perceived hand position towards the rubber hand. We hypothesized that the multisensory integration at rest can be physiologically assessed by combining the RHI paradigm with sensorimotor integration parameters measured by transcranial magnetic stimulation (TMS). <Methods> 8 healthy subjects were recruited. Subjects viewed a rubber hand being stroked by a brush during the application of synchronous (test condition) or asynchronous (control condition) brush strokes on their own unseen hand. For perceptual assessment, the perceived own hand position was verbally reported by the subjects at baseline and after the synchronous or asynchronous condition. Short (SAI) and long (LAI) latency sensory afferent inhibition were used as physiological parameters. Motor evoked potentials (MEPs) were recorded from hand muscles at rest. SAI and LAI were assessed by delivering an electrical stimulation to the index finger followed by TMS to the primary motor cortex (M1) and comparing the conditioned to the unconditioned (without sensory stimulation) MEP amplitudes. SAI and LAI were measured before and immediately after the synchronous or asynchronous brush strokes. <Results> Synchronous strokes induced greater proprioceptive arm

shift than asynchronous strokes, indicating RHI at the perceptual level. At baseline, the ratios of conditioned to unconditioned MEPs for SAI and LAI were 0.79 ± 0.22 and 0.63±0.18, respectively, indicating presence of afferent inhibition. SAI decreased to 0.93±0.31 in the synchronous and to 0.77±0.30 in the asynchronous condition. The ratios for LAI were 0.70±0.24 in the synchronous condition and 0.64±0.19 in the asynchronous condition. <Conclusions> RHI paradigm altered sensorimotor integration by reducing the effects of sensory inputs on M1. Our results may reflect reduced somatosensory processing during RHI and may relate to RHI induced changes at the perceptual and behavioral levels.

Disclosures: R. Isayama: None. **G. Jegatheeswaran:** None. **M. Vesia:** None. **B. Elahi:** None. **C. Gunraj:** None. **L. Cardinali:** None. **A. Farne:** None. **R. Chen:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 724.05/GG32

Topic: D.03. Multisensory

Support: SNF 320030_149561

Title: Random noise stimulation of the cortex: does stochastic resonance enhance central mechanisms of perception?

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Abstract: It has been demonstrated that tactile and visual detection thresholds decrease by adding random noise to the peripheral nervous system, e.g. to the mechanoreceptors of the finger or to retinal input to the visual system (Simonotto et al., 1996, Collins et al., 1996) in accordance to a stochastic resonance (SR) phenomenon. Psychophysical experiments in humans suggest that SR in the visual system can occur in the primary visual cortex (Aihara et al., 2008). In addition, electrophysiological research in cats, where spinal and cortical evoked field potentials (EFPs) were measured, has demonstrated that somatosensory neurons in the central nervous system exhibit SR-like behaviors (Manjarrez et al., 2003). In an EEG (electroencephalography) study in humans, it was shown that when a small amount of tactile random noise was applied to a tactile signal on the fingertip signal-to-noise ratio of the EEG signals decreased for the optimal level of

peripheral noise, which is a characteristic of SR. In the previous mentioned examples the signal and noise were always applied to the peripheral nervous system and not directly to the central nervous system. The question arises as to whether SR only occurs in the peripheral nervous system, or if the same SR-like behavior occurs if noise is added to the cortex. Here subjects performed a visual or a tactile perception task while different levels of noise were added either to the peripheral or central nervous system. Peripheral noise was zero-mean Gaussian noise applied to the index finger or represented on the screen. Central noise was applied via transcranial random noise stimulation (tRNS, 100-640 Hz, intensity varying between 0 and 1.5 mA) with one electrode overlying the primary somatosensory or visual cortex. Subjects had to do a tactile or visual detection task. Preliminary results indicate that peripheral noise induces a much bigger SR effect than central noise.

Disclosures: O.L. Van Der Groen: None. **N. Wenderoth:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

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Topic: D.03. Multisensory

Support: Foundation for Polish Science grant (HomingPlus/2011-4/13)

National Science Centre Poland grant (2012/05/E/HS/03538)

Title: The visual cortex is not exclusively visual, and plays a critical role in tactile Braille reading. fMRI, resting-state fMRI and TMS evidence from sighted Braille readers

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Abstract: According to the standard model, the brain processes sensory input in separate systems: visual cortex, auditory cortex, and so forth. Against this view, several studies showed that tactile Braille reading in the congenitally blind can recruit the visual cortex. Reich et al. (2011) for example showed that tactile reading activates the Visual Word Form Area (VWFA) - a ventral visual region known to develop expertise for visual reading. However, these studies were performed on blind subjects, whose brains undergo large-scale reorganization caused by loss of sight. Their potential implications are therefore not obvious. Here, we asked whether tactile reading can recruit the sighted's visual cortex. 29 subjects (3 male, 26 female, mean age = 29) - special education teachers and students, knowing how to read Braille visually but utterly unable to read it tactilely - were enrolled in a 9-month tactile Braille reading course. At the beginning and at its end, they underwent an fMRI experiment consisting of visual and tactile Braille reading as well as suitable control conditions. Additionally, in both scanning sessions resting-state fMRI (rsfMRI) data were collected. At the end, some subjects were also tested in a Transcranial Magnetic Stimulation (TMS) experiment. Subjects' reached a reading rate of 6+/- 0.72 Braille words-per-minute, with best readers achieving 17 words-per-minute. This, we believe, is the first demonstration that sighted adults can learn to read tactile Braille. Before-training fMRI did not reveal any significant activation for tactile reading vs. control (non-Braille tactile characters) contrast. After the course however, subjects showed enhanced activity for tactile reading in the VWFA, left Inferior Frontal Gyrus (BA 44 and 45) and left Superior Temporal Gyrus. Control conditions' results indicated that these VWFA activations could not be explained by mental imagery. Moreover, in rsfMRI analysis we observed increased functional connectivity between the VWFA and the left primary somatosensory cortex (BA 1 and 2) after learning to read Braille. Finally, TMS applied to the VWFA decreased accuracy of reading words in Braille in a lexical decision task, while such effect was not observed during TMS stimulation of control regions. Our results show that the VWFA - a key node in visual reading network - can reorganize itself in order to take over tactile reading even while receiving visual input. Our study strongly challenges the classic sensory-division-of-labor principle and indicates that some parts of the visual cortex are in fact multimodal regions that process data relevant for a specific task, independent of the sensory modality.

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Poster

724. Multisensory: Cross-Modal Processing in Humans

Location: Halls A-C

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Program#/Poster#: 724.07/GG34

Topic: D.03. Multisensory

Support: JHU Brain Science Institute

JHU Science of Learning

Title: Neural correlates of auditory-tactile integration in meter perception: An EEG study

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Abstract: Previously we reported that auditory and tactile inputs are seamlessly integrated to form the percept of meter in human subjects (Huang, et al. 2012). In this study, we examined the neural correlates of auditory and tactile integration in meter perception using Electroencephalography (EEG) recording. We recorded event related potentials (ERP) from 12 human subjects while they were presented with a unimodal auditory, unimodal tactile, or bimodal sequences. Auditory stimulation was presented through headphones, and tactile stimulation was presented through a tactile vibration stimulator that was placed with subject's left index finger tip. Subjects were also asked to detect whether the last note of some of the sequences (5%) showed changes in amplitude and frequency to assess the attention state of the subject. In the bimodal sequences the auditory or tactile notes signaling whether the meter was duple or triple meter structure were modulated. We report that the EEG signals corresponding to missing notes are similar to those induced by both the metrically important and unimportant notes. The results demonstrate that ERP signals can be induced without actual sensory input (either, auditory or tactile) as long as the "virtual" notes are embedded in a sequence with a valid meter structure. Further more, the EEG components corresponding to bimodal sequences are determined by the modality of the important notes. When the important notes are auditory, the overall EEG waveforms are similar to those induced by unimodal auditory sequences; whereas the EEG waveforms corresponding to bimodal sequences with tactile inputs as the important notes are similar to those induced by the unimodal tactile sequences. The latencies and tonotopical maps of the EEG signals produced by unimodal and bimodal sequences are also modality dependent. Similarities are observed between the signals observed during the unimodal auditory and bimodal conditions when the important notes are carried by the auditory condition, and similarly between unimodal tactile and the bimodal condition with tactile inputs are carrying the important notes. These results suggest that there are neural correlates of auditory and tactile integration of meter perception, which are closely linked to the cognitive aspects of perception and less to the sensory input. The data further suggests that both auditory and tactile modalities can function as the dominant role in the processing of meter information. [This research is supported by JHU BSI and Science of Learning grants.]

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Poster

724. Multisensory: Cross-Modal Processing in Humans

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Program#/Poster#: 724.08/GG35

Topic: D.03. Multisensory

Support: EU-ERC 283567

Title: Scaling the scene: Influence of vestibular and visual depth cues on translational self motion perception

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Abstract: Human self-motion perception is crucial for navigation and goal-directed action. In perceiving self-motion, the brain may combine information from various sensory modalities, including the vestibular and visual systems. To judge the amount of linear motion from optic flow, humans need to scale the egocentric distance of the observed visual objects. Scaling information can be provided by several combinations of cues such as binocular disparity in combination with the vergence angle of the eyes or vestibular information together with motion parallax. In this study, we assessed whether inadequate scaling due to a lack of binocular disparity influences the perception of linear self-motion. Participants were seated on a linear sled embedded in a virtual reality environment. They were subjected to linear motion involving a visual only cue, vestibular only cue or vestibular and visual cues combined. The visual cues could or could not include disparity. Participants performed a two-alternative forced-choice task, indicating which of two sequential displacements was larger. An optimal integration model was fitted on the data using maximum likelihood estimation. Results indicate that passive displacement with motion parallax cues but without disparity cues was overestimated with respect to the same movement with disparity cues. This was also found for the visual only cue conditions. Our results suggest that depth scaling affects the perception of linear motion.

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Poster

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Topic: D.03. Multisensory

Support: T32HD007434

5K08NS064365

Title: Audio-motor interactions in the resting state

Authors: *M. URBIN[1], K. ZINN[2], X. HONG[2], A. CARTER[2];
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Abstract: Strong functional connections exist between auditory and motor systems. These connections are thought to facilitate cross-modal integration of sensory input necessary for rapid, individuated finger movements in skilled musicians. Recent evidence suggests that this audio-motor coupling (AMC) mechanism can be exploited to ameliorate movement-related deficits following hemiparetic stroke. In music-supported therapy (MST), for example, musical instruments are used as a form of motor retraining for the paretic upper extremity. A number of task-evoked fMRI studies have demonstrated AMC in musicians and non-musicians, as well as in stroke patients undergoing MST. The broad purpose of the current study was to explore AMC using resting-state functional connectivity to answer two questions. First, is there any evidence of AMC in the resting state? Second, does musical expertise modulate resting-state connectivity between specific auditory and motor brain regions? To answer these questions, the resting-state fMRI BOLD signal was measured in samples of musicians and non-musicians. Inter-network connectivity with the somatomotor network was evaluated with previously defined resting-state networks, including auditory, dorsal-attention, ventral-attention, language, foveal-visual and peripheral-visual networks. All possible combinations of regions within auditory and somatomotor networks were surveyed to determine which, if any, were modulated by musical training. Results indicated that the somatomotor network was more strongly connected to the auditory network than any other network in both samples. The middle insula and ventral central sulcus were regions of high AMC in both samples. Eleven audio-motor pairs were significantly different between samples with nine exhibiting stronger connectivity in musicians. Five of the pairs linked the right middle insular region (ie, auditory network) with the right superior parietal

lobe (ie, motor network). In summary, these findings indicate that motor brain regions exhibit strong functional connectivity with auditory regions in the resting state, and specific connections between both networks are modulated by musical training.

Disclosures: M. Urbin: None. **K. Zinn:** None. **X. Hong:** None. **A. Carter:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

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Topic: D.03. Multisensory

Support: Department of Radiology and Imaging Sciences, Emory University

Atlanta VAMC

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Title: Resting state functional connectivity networks involved in visual processing of body-part and scene stimuli

Authors: *K. GOPINATH[1], S. LACEY[2], R. STILLA[2], K. SATHIAN[2,3,4,5];
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Abstract: The human brain contains a number of regions specialized for processing particular types of sensory stimuli. We reported earlier that functionally localized regions of occipitotemporal cortex exhibit both common and differential resting state functional connectivity (rsFC) patterns with other neocortical regions (Gopinath et al., SfN Abstracts 2013: 285.01). Here we examined rsFC patterns of brain regions that exhibit *exclusive specialization* for processing body-part and scene stimuli. Resting-state fMRI data were obtained while subjects lay with eyes open in a Siemens 3T TIM Trio magnetic resonance imaging (MRI) scanner. Body-part- and scene-selective regions of interest (ROIs) were identified and exclusively masked using separate functional localizer runs. For each category (e.g. body-part), vectors averaged across each of the ROIs were used to perform whole-brain partial-correlation analysis, controlling for the effects of other ROIs exhibiting exclusive selectivity for the same category. Visual processing of body-parts evoked exclusive body-specific activations in the extrastriate body area (EBA), fusiform body area (FBA), intraparietal sulcus (IPS), and dorsolateral

prefrontal cortex (DLPFC). Among these ROIs, the EBA exhibited exclusive rsFC to the default mode network (DMN) as well as primary motor, occipital and somatosensory cortex. The IPS exhibited exclusive rsFC to the ventral premotor cortex (PMv), somatosensory cortex, pain and attention network areas. The FBA exhibited exclusive rsFC with orbitofrontal cortex and amygdala. The DLPFC exhibited rsFC to attention, pain and fronto-striatal networks. Visual processing of scenes evoked exclusive scene-specific activations (in the occipital place area (OPA), precuneus, retrosplenial cortex (RSC), and parahippocampal place area (PPA). Among these ROIs, the OPA exhibited exclusive rsFC to posterior parietal cortical regions and the frontal eye fields (FEFs). The precuneus exhibited exclusive rsFC to the dorsal premotor cortex and FEFs in addition to dorsal DMN areas as well as somatosensory and pain networks. The RSC exhibited exclusive rsFC to parahippocampal cortex bordering PPA, and ventral DMN areas. The rsFC between body-selective ROIs and the DMN and PMv is consistent with the self-referential and body ownership processes, respectively, that involve these areas. The rsFC maps of scene-selective ROIs revealed the expected links with dorsal frontoparietal association networks. These findings provide interesting insights into the brain networks involved in visual processing of body- and scene-specific stimuli.

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Poster

724. Multisensory: Cross-Modal Processing in Humans

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 724.11/HH2

Topic: D.03. Multisensory

Title: Feeling your touch: Spatial frames of reference and subtypes of mirror-touch synesthesia

Authors: *J. MEDINA, C. DEPASQUALE;
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Abstract: Some individuals experience tactile percepts on their own bodies when observing someone else being touched - a phenomenon called mirror-touch synesthesia (MTS). There is evidence for different subtypes of MTS, based on the coordinate transformations that can be used to map touch between two individuals. We report two experiments that manipulate hand position to examine different subtypes of MTS. In one experiment, we showed over 20 individuals with MTS videos of hands being touched on different surfaces (palmar, dorsal) and in different locations (e.g. index or ring finger). We asked them to report the intensity and location of their

own synesthetic percepts while their own hands were in different postures (palms up, palms down). We found two distinct subtypes of MTS. A subset of individuals demonstrated *somatotopic* MTS, in which they consistently reported synesthetic percepts on the same location on the skin surface that was touched in the video. A second group, however, experienced mirror-touch percepts based on an *external* reference frame, reporting illusory percepts in the same spatial location as in the video (e.g. with their palms up, viewing dorsal stimulation on the video ring finger produced palmar sensation on their own index finger). In a second experiment, we examined the spatial location of synesthetic percepts in a tactile congruency task. Two tactile stimulators were placed on the dorsal fingertip of the index and ring finger of either hand in mirror-touch synesthetes and controls. Participants viewed videos of a left or right hand from different perspectives (1st person or 3rd person) being touched (or not touched) on the index or ring finger. While watching the video, participants were presented with no stimulation or a tactile stimulus to either finger, and were asked to quickly respond (via foot pedals) to where they were touched. Compared to controls, mirror-touch synesthetes were significantly slower to respond and made significantly more errors on this task. Although both groups demonstrated a spatial congruency effect, this effect was significantly greater in mirror-touch synesthetes. Finally, somatotopic mirror-touch synesthetes demonstrated a *somatotopic* congruency effect (slower responses when the video and actual stimulation were on different fingers), whereas external mirror-touch synesthetes demonstrated a *spatial* congruency effect (slower responses when the video and actual stimulation were on different sides of space). We discuss the potential mechanisms that cause different subtypes of mirror-touch synesthesia.

Disclosures: J. Medina: None. **C. DePasquale:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

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Program#/Poster#: 724.12/HH3

Topic: D.03. Multisensory

Title: The role of therapy ball seating on classroom performance: Understanding the physiological mechanisms

Authors: *M. E. BURGOYNE, C. J. KETCHAM;
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Abstract: The academic success of students is a major concern in American culture; thus, there is constant pressure for new discoveries that can improve education. Many classrooms are beginning to substitute standard chairs with therapy balls, which help to improve students' focus and classroom performance, according to teacher and student reports. Therapy balls are a type of heightened sensory tool, which are often used in physical and occupational therapy as a strategy for individuals with learning or sensory differences. Heightened sensory tools increase the sensory information that the brain receives and have been effective at improving attention and classroom performance. However, no studies explain why these tools are successful. An observational study indicated that attention and academic task improved with the use of therapy balls compared to standard chairs and that the effect of vestibular and proprioceptive input should be further investigated in a laboratory setting. Researchers performed an experiment with elementary school age participants (N = 20) to examine the effect of heightened sensory stimulation on the performance of functional school tasks and standard balance tasks. Subjects performed math and comprehensive reading tests during seating on a standard chair, seating with increased vestibular input, and seating with increased proprioceptive input. They also completed static balance tasks with eyes open/closed on a firm/foam surface using the Biodex Balance system. Preliminary results suggest that with the utilization of therapy balls, school function either is the same or better compared to a standard chair. In addition, balance measures improve when proprioceptive and visual input are occluded or distorted after spending time on the stability ball. This research has the potential to help develop specific and evidence based training for teachers and students on the appropriate strategies to use alternative seating in a classroom setting.

Disclosures: **M.E. Burgoyne:** None. **C.J. Ketcham:** None.

Poster

724. Multisensory: Cross-Modal Processing in Humans

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Title: fNIRS and fMRI signals are concordant during a bipedal motor task

Authors: *J. A. NOAH[1], Y. ONO[3], Y. NOMOTO[3], S. SHIMADA[3], A. TACHIBANA[4], X. ZHANG[1], S. BRONNER[5], J. HIRSCH[1,2];
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Abstract: An often quoted benefit of fNIRS is that the technique allows for studying neural mechanisms of complex, naturalistic behaviors that are not possible using the constrained environment of fMRI. However, it has recently been shown that fNIRS can accurately record functional brain activities equivalent to those concurrently recorded with fMRI for classic psychophysical tasks and simple finger tapping paradigms. Our goal was to extend the findings of previous studies that have shown high correlation between concurrently recorded fNIRS and fMRI signals to compare neural recordings obtained in fMRI to those separately obtained in naturalistic fNIRS experiments. Utilizing a multimodal motor-sensory paradigm that is compatible with both fMRI and fNIRS, we tested a hypothesis that there would be concordance between fMRI and fNIRS signals recorded from cortical regions involved in multisensory integration. We used a modified version of the dance video game, Dance Dance Revolution (DDR) as a multimodal decision-making task, and set the superior and middle temporal gyri as the regions of interest. Modifications were made to software and hardware for compatibility with each technique. Forty two subjects (fMRI = 16 fMRI, fNIRS = 26) underwent the scanning procedure. The results of the study show it is possible to replicate the findings of fMRI using fNIRS in a naturalistic task with high correlation (correlation coefficient = 0.78, $p = 0.0008$) between signals acquired by fMRI in the middle and superior temporal gyri. These results show that fNIRS can be used for imaging naturalistic, full-body activities and behaviors with high correlation to fMRI imaging paradigms which utilize a reduced-world environment. Further development of fNIRS imaging may provide insight to the neural mechanisms of the benefits of training or prevention programs targeted at treating neurodegenerative diseases, such as Parkinson's disease, dementia, or others with magnetic susceptibility which are contraindicated for fMRI scanning.

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Poster

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Program#/Poster#: 724.14/HH5

Topic: D.03. Multisensory

Title: The effect of naturalistic versus robotic speech on multisensory processing: applications for individuals with autism spectrum disorder (ASD)

Authors: *F. SARTORATO[1], L. PRZYBYLOWSKI[1], D. K. SARKO[2];
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Abstract: The perception of stimuli through more than one sensory modality results in a wide range of behavioral benefits including improved stimulus detection, increased response accuracy, and faster reaction times. Although these enhancements have been demonstrated using both simple and complex multisensory stimulus pairs, they have not yet been extended to naturalistic versus robotic speech. This application would be particularly useful in assessing multisensory integration in individuals with autism spectrum disorder (ASD), for whom social robots are increasingly utilized as tools for social skills and communication therapies by coupling social interaction with intrinsic reinforcers and motivators. Robots generate increased engagement and attention in autistic subjects and confer the added benefit of occupying a niche between inanimate toys (not eliciting social behaviors) and animate human beings (whose social cues can confuse and distress autistic individuals). Given the use of robots as therapy tools in individuals with ASD, we aimed to test the effect of naturalistic versus robotic speech on the size of the temporal binding window (a probabilistic depiction reflecting the likelihood that two stimuli from different modalities will be perceptually bound as a unified, synchronous event). As the first step in this process, we developed audiovisual stimuli using naturalistic human speech compared to robotic speech, testing responses at various stimulus onset asynchronies (SOAs). We predict that a narrower temporal binding window would be seen under naturalistic speech conditions in normal subjects, with robotic speech potentially conferring greater benefits in audiovisual processing for autistic subjects. Stimuli will be developed and tested in normal subjects with the goal of comparison to clinical populations in which multisensory processing is impaired, particularly individuals with autism spectrum disorder for whom social robots may confer effective therapeutic outcomes.

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Poster

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Topic: D.03. Multisensory

Support: ERC grant agreement number 295673

Title: Investigating implicit crossmodal decoding of body-voice emotion using multivariate pattern analysis

Authors: *R. WATSON, B. DE GELDER;
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Abstract: Basic emotional states (e.g., anger, happiness) can be well recognised regardless of whether they are conveyed by the body or voice. Furthermore, we rarely perceive affect from each sensory modality alone, but rather simultaneously, and combine this into a multimodal percept. The purpose of the presented study is to investigate, using fMRI, how and where affective information from the body and voice is represented and combined in the brain, when participants were not explicitly engaged in emotion evaluation. Participants underwent a slow-event related 3T fMRI scan while being presented with dynamic affective video (body) and audio (vocal expression) clips (expressions: angry, fear, happy, neutral). We utilised four emotions (anger, fear, happy, neutral). At the same time, participants performed an unrelated, distractor task, which occurred in 12.5% of trials. Using multivoxel pattern analysis, we searched for regions that could not only discriminate between different affective states within modality (e.g., body - body), but also across modality (i.e., body - voice). Specifically, we used a multivariate Feature Elimination procedure to select sets of voxels with the highest discriminative power in the whole brain SVM classification. With this procedure, it is possible to detect patterns of brain activities distributed across a wide-spread network in the brain. For each condition pairing, we trained on 50% of trials and tested on the remaining 50%. We further performed 50 ‘inner permutations’ (i.e., shuffling trials within each condition) so to properly evaluate each voxel’s real contribution to classification (i.e., the voxel weight); and 30 ‘outer permutations’ (i.e., shuffling trials across conditions) to establish the correct baseline categorisation score. We then searched for voxels in which discrimination was above chance (i.e., 2 standard deviations above the baseline score). Initial results highlight a network of regions involved in intramodal and crossmodal decoding of body and vocal emotion. Above chance discrimination of body emotion was observed in fusiform gyrus, posterior cingulate, posterior superior temporal sulcus/gyrus, and inter-parietal sulcus, and voice emotion in mid-anterior superior temporal sulcus/gyrus, inter-parietal sulcus, thalamus, amygdala and inferior frontal gyrus. Crossmodal decoding was observed in the inter-parietal sulcus and superior temporal sulcus, along with the cerebellum. These results reveal amodal representations of emotion in

brain areas previously implicated in integration of multisensory signals, even when affect is not being explicitly evaluated.

Disclosures: **R. Watson:** None. **B. de Gelder:** None.

Poster

725. Retinal Circuitry

Location: Halls A-C

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Program#/Poster#: 725.01/HH7

Topic: D.04. Vision

Support: NIH

Agency for Science, Technology and Research (A*STAR), Singapore

Title: Development of dendrites in a direction-selective ganglion cell in the mouse retina

Authors: ***J. LIU**[1,2], J. R. SANES[1];
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Abstract: Dendritic shape and position are important determinants of neuronal connectivity and function. To examine dendritic morphogenesis, we focus on a retinal ganglion cell (RGC) type in the mouse retina that responds preferentially to upward motion in the visual field; it is called J-RGC for its selective expression of the adhesion molecule JAM-B (Kim et al., Nature 2008). Unlike most RGC types whose dendrites ramify from the cell body in all directions, J-RGCs have highly asymmetric dendrites that project ventrally, coinciding with their direction selectivity. They laminate at the outermost part of the inner plexiform layer (IPL) of the retina, conferring OFF-response properties upon J-RGCs. We are asking how J-RGC dendrites acquire their asymmetry, laminar restriction and synaptic distribution. (1) Asymmetry. Using transgenic reporters to specifically label J-RGCs, we show that their dendritic outgrowth is initially unbiased but become ventrally asymmetric during the first two postnatal weeks. This development of asymmetry progresses across the retina in a dorsoventral wave. Eventually, all J-RGCs in the equatorial retina become markedly asymmetric, but J-RGCs at the dorsal and ventral margins of the retina remain symmetric. These findings suggest a model in which a dorsoventrally graded cue within the retina patterns J-RGC dendrites into a ventrally asymmetric arbor. (2) Laminar specificity. J-RGC dendrites become restricted to the outermost part of the

IPL by eye opening (Kim et al., J. Neurosci. 2010). By mapping their laminar positions over development, we show that J-RGC dendrites attain laminar specificity very early and independently of their asymmetry. This implies that distinct mechanisms operate within different dimensions of J-RGC dendritic development. (3) Synaptic distribution. We introduced fluorescently-tagged synaptic markers, gephyrin and PSD95, to mark inhibitory and excitatory synapses respectively within J-RGCs. Initial results suggest that excitatory synapses are more uniformly distributed through the arbor than inhibitory ones. (4) Molecular mechanisms. We are currently using RNA-seq of dorsal and ventral retinas, taken at ages when J-RGCs are most rapidly turning asymmetric, to identify extrinsic mediators of asymmetry. Similarly, we are using RNA-seq to profile gene expression in developing J-RGCs to identify intrinsic determinants of dendritic development. We are also using targeted mutants to assess the role of these candidate mediators, including JAM-B itself. Together, these experiments may reveal general principles underlying dendritic patterning.

Disclosures: J. Liu: None. **J.R. Sanes:** None.

Poster

725. Retinal Circuitry

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Title: Roles of protocadherin-mediated self-avoidance and self/non-self discrimination in retinal circuit function

Authors: *D. KOSTADINOV[1], J. R. SANES[2];
[1]Dept of Neurobio., [2]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Many types of neurons pattern their dendrites and axons through a process known as self-avoidance, in which neurites of a single neuron repel each other while interacting freely with neighbors of the same type. Mammalian retinal starburst amacrine cells (SACs) form planar, radially symmetric dendritic arbors in which branches emanating from one SAC seldom cross each other and thus self-avoid. However, SAC dendrites overlap with and make synapses on

dendrites of neighboring SACs, implying that individual cells can distinguish self from non-self. We recently demonstrated that the gamma-protocadherins (Pcdhgs), a set of 22 cell adhesion molecules, mediate dendritic self-avoidance and self/non-self discrimination in mouse SACs. Deletion of all Pcdhgs leads to loss of self-avoidance within SACs but maintenance of overlap between SACs. In contrast, rescue of the mutant with a single Pcdhg (endowing all SACs with the same Pcdhg repertoire) restores self-avoidance and also decreases overlap between SACs - as if they mistake dendrites of neighboring SACs for their own (Lefebvre, Kostadinov et al., Nature, 2012). Here, we used patch clamp electrophysiology to learn how self-avoidance and self/non-self discrimination affect SAC function. Individual SAC dendrites are electrically isolated directionally selective subunits that make GABAergic synapses on direction-selective retinal ganglion cells (DSGCs), endowing DSGCs with their direction selectivity. The radial morphology of SACs and inhibitory synapses between SACs are also thought to contribute to the direction-selectivity inhibition that SACs provide to DSGCs. Basic physiological properties of SACs and DSGCs in mutant and "single-isoform" retinas were normal. However, SACs lacking Pcdhgs were interconnected at higher frequency and SACs expressing a single isoform were connected at lower frequency than control SACs. In the absence of Pcdhgs, SACs formed autapses, as if they could not distinguish their own dendrites from those of neighboring SACs. DSGCs in mutant retinas were less direction-selective and had a broader range of directional tuning than in control retinas. Rescue of self-avoidance with a single Pcdhg isoform restored the directional tuning preference but not strength of directional responses of DSGCs. Thus, although we cannot exclude other roles of Pcdhgs, we suggest that self-avoidance and self/non-self recognition in SAC dendritic arbors are essential for proper computation of direction-selectivity in retina.

Disclosures: **D. Kostadinov:** None. **J.R. Sanes:** None.

Poster

725. Retinal Circuitry

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Banting Postdoctoral Fellowship

Title: Sidekick 2 directs formation of a non-canonical OFF pathway required for function of an object motion selective circuit

Authors: *A. KRISHNASWAMY[1,2], M. YAMAGATA[1,2], X. DUAN[1,2], J. R. SANES[1,2];
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Abstract: In the retina, ~30 types of retinal ganglion cells connect with interneurons to form circuits that extract specific features from the visual scene. A general consensus is that RGC feature selectivity depends on the interneuron types (amacrine and bipolar cells) that innervate it. The interneuron connectivity received by most RGC types is poorly understood. To analyze this connectivity and elucidate its developmental determinants, we implemented an optogenetic approach. We cross transgenic mice that express cre recombinase in specific interneuron types to mice that express YFP in specific RGC types. We target channelrhodopsin (ChR2) to the interneurons in these mice, activate ChR2 with 2-photon illumination, and record from RGCs. Here, we measured connectivity of several interneuron types to an object motion selective RGC called W3B that we recently characterized (Zhang, PNAS, 2012). W3B is a local motion detector and its light responses lag that of many other RGC types. This lag allows surround inhibition to silence W3B during most common visual stimuli; they thus respond selectively to objects moving locally in the receptive field center. Amacrine cells that express vesicular glutamate transporter 3 (VGlut3 ACs) were monosynaptically connected to W3B RGCs via glutamatergic synapses. To assess the contribution of this connectivity to W3B responses we targeted diphtheria toxin receptor to Vglut3ACs and selectively ablated them by administering diphtheria toxin. The loss of VGlut3 ACs ablated the OFF response in W3Bs, indicating that VGlut3 ACs account for part of the delayed excitatory light responses on these RGCs. We discovered that both VGlut3 and W3B cells express the homophilic adhesion molecule Sidekick 2, the mammalian orthologue of a gene implicated in retinal connectivity in chicks (Yamagata, Nature, 2008). VGlut3 ACs were connected more strongly to W3Bs than to Sidekick 2-negative RGC types that co-stratified with W3B. Moreover, loss of Sidekick 2 in knockout mice reduced connectivity between VGlut3 and W3B cells by >90%. Importantly, this decrease was specific to VGlut3-W3B connections: connectivity of VGlut3 ACs to other RGCs and of other interneurons to W3B RGCs were unaffected. Loss of VGlut3 AC synapses, either from ablation or Sidekick 2 mutation, resulted in a dramatic loss of OFF responses in W3B RGCs, consistent with the role of VGlut3 ACs as the primary channel for OFF input on these RGCs. Taken together our results indicate that VGlut3 ACs form a non-canonical OFF pathway on W3B RGCs that is constructed by Sidekick-directed synapse formation.

Disclosures: **A. Krishnaswamy:** None. **M. Yamagata:** None. **X. Duan:** None. **J.R. Sanes:** None.

Poster

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Damon Runyon Cancer Research Foundation

Title: Cadherin 13 patterns synapses at mammalian cone photoreceptor terminals

Authors: *S. SARIN, M. YAMAGATA, J. R. SANES;
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Abstract: In mammalian photoreceptors, neurotransmitter release occurs from synaptic ribbons, at which rows of synaptic vesicles are tethered to protein-rich plate-like structures. Cone photoreceptors bear multiple ribbons, each apposed to a triad of interneuronal processes, one bipolar and two horizontal cells. All ribbon synapses are confined to a central domain within the large cone terminal, called a pedicle. Although several molecules have been identified as structural components of the ribbon, the molecules that affect their placement remain unexplored. Here we describe a role for an atypical cadherin, Cdh13, in the development of mammalian cone photoreceptor synaptic clusters. We generated a Cdh13-inducible Cre recombinase knock-in/knock-out mouse line to analyze Cdh13 expression and function. Using *in situ* hybridization and a reporter line, we show that Cdh13 is expressed in both photoreceptors and their interneuronal synaptic partners, horizontal cells. We observed Cdh13 homozygous mutants and found that ribbons in the cone photoreceptor no longer cluster, but rather fill the entire terminal. This ribbon mislocalization is evident by postnatal day 6, as the first synaptic contacts are being made between photoreceptors and horizontal cells, and persists throughout adulthood. Electron microscopy reconstructions confirm that Cdh13 mutants exhibit ribbons with >2 fold greater dispersion. The defect is specific in that there is no significant change in the numbers of photoreceptors or their interneuronal synaptic partners or in the total number of ribbons per terminal. The laminar pattern of synapses and cell bodies in the outer retina also is not disrupted. Taken together, these results indicate that Cdh13 patterns release sites within cone terminals. More generally, mechanisms underlying the patterning of release sites within a single axon remain underappreciated features of neuronal organization. As Cdh13 has previously been shown to bind homophilically in trans, we are testing the hypothesis that Cdh13-mediated

interactions between photoreceptors and horizontal cells limit the potential space for synapse formation. (Supported by the NIH and Damon Runyon Cancer Research Foundation)

Disclosures: S. Sarin: None. **M. Yamagata:** None. **J.R. Sanes:** None.

Poster

725. Retinal Circuitry

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Support: NIH Grant EY018625

NIH Grant EY022369

Title: Phosphorylation by PKCα in retinal rod bipolar cells

Authors: C. W. MORGANS, W.-H. XIONG, M. TEKMEN CLARKE, *R. M. DUVOISIN; Physiol. & Pharmacol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Protein kinase C alpha (PKCα) is abundantly expressed in rod bipolar cells in the retina, yet the physiological role of PKCα in these neurons is not well understood. Immunohistochemistry and western blotting results suggest that PKCα expression in the rod bipolar cell is activity dependent. In the light-adapted retina, PKCα immunofluorescence is brighter in the tips of the rod bipolar cell dendrites and in the axon terminals compared to dark-adapted tissue. Supporting activity-dependent regulation of PKCα expression, PKCα protein was dramatically reduced in the TRPM1 knockout mouse retina, in which ON-bipolar cells are unresponsive to light. Electroretinogram (ERG) recordings demonstrated that genetic deletion of PKCα in mice has a dramatic effect on the scotopic ERG b-wave, including a larger peak amplitude, longer implicit time and broader width of the b-wave. The effect of PKCα deletion on the ERG b-wave was enhanced at brighter flash intensities. The photopic ERG was unaffected, consistent with the lack of detectable PKCα in cone bipolar cells. Immunofluorescent labeling of retina sections with an antibody against phospho-serine revealed that the outer plexiform layer (OPL) is a major site of serine phosphorylation in the mouse retina. Punctate labeling similar to that obtained with antibodies to mGluR6 was observed, indicating the presence of phosphorylation sites in the rod bipolar cell dendritic tips. Phospho-serine immunofluorescence in the OPL was reduced in the PKCα knockout retina, indicating that PKCα is required for

maximum serine phosphorylation at this site. In conclusion our results suggest that PKCα plays an important modulatory role in the rod bipolar cell, regulating both the peak amplitude and temporal properties of the rod bipolar cell by phosphorylating serine residues on target proteins in the rod bipolar cell dendrites.

Disclosures: C.W. Morgans: None. **R.M. Duvoisin:** None. **W. Xiong:** None. **M. Tekmen Clarke:** None.

Poster

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Support: Grant SENRYAKU 1001034 from the Ministry of Education, Culture, Sports, Science & Technology in Japan.

Title: Immunohistochemical localization of histamine receptor subtypes in the gerbil retina

Authors: H. IMADA[1], K. SAKAI[2], *E.-I. MIYACHI[3];
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Abstract: Histamine is recognized as one of neurotransmitters and of neuromodulators in the central nervous system. And histamine is known to act on four major types of G-protein-coupled receptors: histamine H1 receptor (H1R), histamine H2 receptor (H2R), histamine H3 receptor (H3R) and histamine H4 receptor (H4R). In the mammalian retinal cells, histamine receptors have been reported to be present. In the Society for Neuroscience - Neuroscience 2011, we reported that H1R, H2R and H3R exist on the gerbil retinal ganglion cells (RGCs). In order to confirm the presence of histamine communication system in the gerbil retina, we made experiments using the methods of immunohistochemical analyses. The animals were perfused intra-cardially with a mixture of 4% paraformaldehyde in 0.1M sodium phosphate buffer. After the removed eyeballs were frozen with the liquid nitrogen, they were cut with section of 16 μm using a cryostat. The sections were examined by ABC (avidin-biotin-peroxidase complex) immunocytochemical staining method. We have found H1R, H2R, H3R and H4R in gerbil retinae. We examined the localizations and developmental changes of the four types of histamine

receptors from 1 to 350 postnatal days in the gerbil retinae. H1R, H2R and H3R were expressed in RGCs. However, the expressions of H2R and H3R became maximum from 14 to 21 postnatal days. H4R was expressed in the outer plexiform layer (OPL) and at the outer segments of photoreceptors. Since the gerbil opens the eyes at 3 weeks old, it is considered that the H2R and H3R play some specific roles at the formation of the early visual system. On the other hand, H1R were expressed through the retinal maturation. Therefore, H1R seems to contribute to retinal visual information transmission in adults. Histidine decarboxylase, which produces histamine from histidine, also expressed in RGCs, and moreover, each of histamine receptors and histidine decarboxylase were co-localized in the same RGCs. These findings suggest that RGCs interact with each other via histamine. Histamine may be one of the important neurotransmitters and/or neuromodulators in the visual information processings of the mammalian retina.

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Poster

725. Retinal Circuitry

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Title: A morphometric and transcriptional analysis of retinal ganglion cells in the prenatal macaque monkey

Authors: *K. D. MURRAY[1], D. A. VAN DER LIST[2], W. M. USREY[2];
[1]Psychiatry & Behavioral Sci. and Ctr. for Neurosci., Univ. California Davis, Davis, CA; [2]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: In vertebrates, visual information is conveyed to the central nervous system along parallel streams generated by distinct sets of retinal ganglion cells (RGCs). In adult macaque, RGC subtypes are characterized on the basis of differences in their function, morphology and axonal projections, but when these distinctions emerge during development is not known. Unlike mouse, cat or ferret, macaque retinogeniculate projections mature prenatally and are morphologically adult-like by approximately gestational day 145 (GD145), almost 20 days

before birth. Whether other distinguishing characteristics of RGCs are similarly mature prenatally is unknown. To address this we performed a morphometric analysis of retinal ganglions cells at GD145-147 and mapped the results onto a comprehensive database of over 300 adult RGCs using four cardinal morphological parameters (soma size, dendritic field area, number of branches and location of dendritic stratification within the inner plexiform layer). Prenatal RGCs were filled with Alexa Dye following patch clamp recording and cellular reconstruction was performed from confocal images using MicroBrightfield Neurolucida software. Prenatal RGCs segregated into either "ON" or "Off" functional types as well midget, parasol, wide field, large sparse and bistratified cells. These results suggest that RGC dendritic arbors and somal sizes are adult-like prenatally, similar to their axonal projections. To investigate whether other cellular and molecular characteristics of RGCs were similarly mature prenatally we performed a genome-wide transcriptional analysis. Laser microscopy captured RGCs were processed for Affymetrix microarray analysis and developmentally regulated genes were identified by Genespring software analysis. Over 1200 genes were found to be up- or down-regulated from GD145 to adult (2-fold or greater, $p<0.05$). We interrogated the functional relatedness of these genes using ontological and functional interaction databases (DAVID and Ingenuity Pathway Analysis software). The most significant functions associated with genes up-regulated during development were linked with membrane associated proteins (e.g. "integral to membrane"), clathrin mediated endocytosis and cell-cell signaling. In contrast, the most significant functions associated with developmentally down-regulated genes were overwhelmingly related to regulation of transcription and protein translation. Taken together these observations suggest that macaque RGCs are cytoarchitectural mature by birth but that cellular changes focused on production of membrane linked signaling proteins continue to develop.

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Poster

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Title: Multiple VIP-cre expressing amacrine cells in the mouse retina

Authors: *N. C. BRECHA[1,2], A. R. RODRIGUEZ[1], A. SOLOMON[1], L. PÉREZ DE SEVILLA MÜLLER[1], H. VUONG[1], K. SHEETS[1], B. WONG[1], S. BARNES[3];
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Abstract: Amacrine cells are a large and heterogeneous group of inhibitory interneurons that form retinal microcircuits to process visual information in the inner retina. To define the morphological and functional properties of the amacrine cell types expressing vasoactive intestinal peptide (VIP) in the mouse retina, we generated VIP-tdTomato and VIP-Brainbow mouse lines. The majority of VIP-tdTomato fluorescent cell bodies were in the inner nuclear layer (INL) and fluorescent processes were distributed to laminae 1, 3, 4 and 5 of the inner plexiform layer (IPL). There were also occasional VIP-tdTomato cell bodies in the ganglion cell layer (GCL). Overall cell density in the INL was ~700 cells/mm^2. In the GCL cell density was very low and some regions lacked fluorescent cells. All tdTomato fluorescing cells contained VIP immunoreactivity, and all VIP immunoreactive cells contained tdTomato fluorescence. Every VIP-tdTomato fluorescent cell also contained HPC-1 and GABA immunoreactivity, and no VIP-tdTomato fluorescent cells expressed the ganglion cell marker, RBPMS. These findings indicate the VIP-fluorescent cells are amacrine cells. Brainbow fluorescence was confined to individual cells with well-defined processes; one group of cells, VIP-1, mainly ramified in laminae 3 and 4 of the IPL, and a second group of cells, VIP-2, ramified in lamina 1 and 5 of the IPL. Neurobiotin labeling of VIP-tdTomato cells in the INL confirmed these two cell types and revealed coupling to numerous other amacrine and ganglion cells. VIP-tdTomato cells in the GCL showed no tracer coupling. We performed whole-cell patch clamp recordings in retinal slices under voltage- and current-clamp, using Lucifer yellow-filled pipettes to label the recorded VIP-tdTomato cells and permit visualization of their dendritic ramifications. Voltage clamp recordings showed the presence of multiple K^+ current types including TEA-sensitive delayed rectifier K_v, BK, and A-type. TTX-sensitive Na^+ currents were observed, but many VIP-tdTomato cells had no or very small Na^+ currents. Most cells lacked action potentials under current clamp, producing spikelets at resting potentials and oscillations in response to depolarizing current injection. Verapamil blocked sustained L-type Ca^{2+} currents. We have identified a novel amacrine cell population consisting of two major cell types in the INL that are characterized by VIP expression. These findings provide the foundation for functional studies to define the roles of these amacrine cells in visual information processing.

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Poster

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Indiana Wesleyan University Hinds Fellowship

Title: Bicarbonate-dependent, potassium-induced increase in proton flux at the endfoot of isolated Muller cells of the tiger salamander

Authors: D. SWYGART[1], M. OSBORN[1], B. SKINNER[1], E. NAYLOR[1], R. KAUFMAN[1], B. WILLIAMS[1], B. TCHERNOOKOVA[2], R. P. MALCHOW[2], *M. A. KREITZER[1]; [1]Biol., Indiana Wesleyan Univ., MARION, IN; [2]Biol. Sci., Univ. of Illinois at Chicago, CHICAGO, IL

Abstract: Within the retina and the broader CNS, synaptic transmission is extremely sensitive to minute changes in pH. A growing number of studies suggest that regulation of extracellular pH plays an important role in shaping neuronal communication.. Tightly regulated levels of HCO_3^- are an understated contributor to extracellular pH. Levels of this extracellular pH buffer are impacted by blood flow, CO_2 levels, as well as the expression and activity of HCO_3^- transporters and the enzyme carbonic anhydrase. Previous work (Newman, 1996) detected the presence of HCO_3^- transporters and carbonic anhydrase on radial glia (Müller cells) that span much of the overall thickness of the retina. The Müller cell plays a primary role in regulating many aspects of the retinal environment, such as ion, H^+, and neurotransmitter levels, and an active role in the release of gliotransmitters. Newman's observations suggested a HCO_3^--dependent mechanism by which high extracellular K^+ acidified the extracellular environment at their endfoot. This mechanism could be important for H^+ clearance to the vitreal surface of the retina as well as in regulating neuronal communication during times of increased neuronal activity. Our findings, using a novel ultrasensitive H^+-selective self-referencing system in combination with a newly develop CO_2 chamber, corroborate these previous studies. The self-referencing system utilizes a

H^+-selective microelectrode that records measurements from a near and a far point from a cell in order to obtain a differential pH value 1000 times more sensitive than a stationary pH-selective electrode. Our work suggests that Müller cells, isolated from tiger salamander retina, respond to increased K^+_o with an extracellular acidification at the endfoot. This acidification can be abolished when extracellular Na^+ or HCO_3^- is removed or in the presence of the HCO_3^- transport antagonist, DIDS. This DIDS-sensitive pH regulatory mechanism could also evoke large extracellular alkalinizations and acidifications when the bathing media was changed between a low Na+ and normal Na+ environment, respectively. These findings extend previous work strongly implicating an important role for HCO_3^- in shaping extracellular pH by Müller cells in the retina. They warrant future studies to characterize whether these bicarbonate-mediated alterations in pH contribution in a significant wayto the processing of visual signals in the retina.
*Newman EA. Acid Efflux from Retinal Glial Cells Generated by Sodium Bicarbonate Cotransport. *J Neurosci*. 1996

Disclosures: **D. Swygart:** None. **M. Osborn:** None. **B. Skinner:** None. **E. Naylor:** None. **R. Kaufman:** None. **B. Williams:** None. **B. Tchernookova:** None. **R.P. Malchow:** None. **M.A. Kreitzer:** None.

Poster

725. Retinal Circuitry

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Title: Modulation of TRPM1 channel in ON bipolar cells by G-protein subunits

Authors: Y. XU[1], S. YANG[1], C. CHOI[2], L. BIRNBAUMER[2], *N. VARDI[3];
[1]GHM Inst. of CNS Regeneration,, Jinan Univ., Guangzhou, China; [2]Natl. institute of Envrn. Hlth. Sci., Research Triangle Park, NC; [3]Univ. Pennsylvania Sch. Med., PHILADELPHIA, PA

Abstract: In darkness, glutamate released from photoreceptors hyperpolarizes retinal ON bipolar cells by activating the heterotrimeric G-protein Go and closing TRPM1 channel. While the basic elements of the cascade are known, the detailed interactions and the cascade progression are far from understood. Here we asked which subunit, Gαo1 or G βγ, mediates the channel closure. Whole-cell patch recordings were made from mouse rod bipolar cells clamped at -60 mV. Retina

was perfused with Ames solution including strychnine and picrotoxin (to block Cl- channels). Control pipette solution included cesium (to block K+ channels), BAPTA (to buffer Ca2+) and ATP. The behavior of the TRPM1 channel was tested by dialyzing cascade modifiers through the recording pipette. The retina was either dark or light adapted and a light pulse (ON or OFF) was given every 35 seconds. The holding current and light responses were compared over time between different dialyzed solutions. Under light adaptation, dialyzing GTP-γ-S (25 μM to 50 μM) quickly decreased the basal current and diminished the light OFF response, confirming that activated G protein cascade closes the channel. But when Gαo1-GTP (100 nM) was dialyzed into bipolar cells (n=18), there was no change in either basal current or light OFF responses, indicating Gαo1 does NOT close the channel. Dialyzing GDP-β-s (500 μM, n=3) to de-activate Gαo1 didn't cause any change either. Under dark adaptation, dialyzing Gαo1-GTP (n=25) increased the holding current significantly. De-activating Gαo1 with GDP-β-S (n=15) removed the effect, leaving the holding current as stable as in control cells (n=20). Similarly, dialyzing constitutively active mutant of Gαo1 failed to cause any change in the holding current (n=22). These data suggest that wild type Gαo1 opens the channel. To test the effect of Gβγ, phosducin (9 μM), a scavenger of this dimer, was dialyzed under dark adaptation. The basal current increased significantly (n=5) and the light ON response increased slightly as well. A mutant phosducin that lost the ability to bind Gβγ did not change the current (n=3). This is consistent with Gβγ closing the channel: by scavenging Gβγ with phosducin, the channel opens to allow more cations flow into the cell. Overall, our results suggest that inactive Gαo maintains the channel open, while free Gβγ closes the channel.

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Poster

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EMBO ASTF 359-2012

Title: Subunit specific expression of glycine receptors in mouse retinal ganglion cells

Authors: *C. ZHANG[1], M. A. MCCALL[2];
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Abstract: Different types of retinal ganglion cells (RGCs) are selectively wired within retinal circuits to extract specific information from the visual scene. The diversity of both inhibitory retinal interneurons and receptors should contribute to the differential RGC visual coding. There are four glycine receptor (GlyR) α subunit isoforms (α1, 2, 3 and 4). Together they mediate half of the inhibition in the inner plexiform layer (IPL). The subunits have different kinetics and show broad, albeit differential expression across the IPL, suggesting that subunit specific inhibitory circuits should exist. A lack of subunit specific pharmacological agents and subunit selective cell class knockouts has made it difficult to evaluate this question. At present, only the most proximal effects of glycinergic inhibition in the retinal circuit have been evaluated. Except for the A-type RGCs, which express GlyRα1, the isoform of GlyR α subunits expressed across different RGC morphological or functional classes remains largely unknown. We have used adeno-associated virus (AAV) to perform RNAi in A-type OFF RGCs to knockdown expression of GlyRα1, eliminate the majority of their glycinergic input and directly investigate the role of GlyRα1-mediated inhibition in A-type RGCs with the remaining glycinergic circuitry intact. To understand the role of each subunit in retinal processing, we have characterized the GlyR α subunits expressed in a variety of different RGC morphological and functional subtypes, using the reporter mouse line PvalbCre × ThyStp-EYFP (PV). In these mice, eight RGC subtypes (PV0-7) express YFP. Specifically, we analyzed: glycinergic spontaneous inhibitory postsynaptic currents (sIPSCs), using whole cell patch clamp recordings and expression of each GlyR α subunit on RGC dendrites, using immunohistochemistry. PV1, 2, 4, 5 and 6 RGCs, which represent ON and OFF RGCs as well as transient and sustained responses, express GlyRα1. In light of our more extensive data on PV5 (A-type OFF), this finding indicates that GlyRα1 plays a general role via a feedforward inhibition that may contribute to the fidelity of visual signaling properties across RGCs. In contrast, PV0 (ON-OFF direction selective RGCs) express GlyRα2 and PV7 (JAM-B RGCs) express GlyRα3. These results imply distinct glycinergic inhibition and potentially differential roles in visual coding. Our previously published results (Nobles et al., 2012) indicate that their roles should be selective to temporal and not spatial signaling. In conclusion, the expression patterns of GlyR α subunits suggest subunit specific glycinergic regulation of different types of RGCs.

Disclosures: C. Zhang: None. **M.A. McCall:** None.

Poster

725. Retinal Circuitry

Location: Halls A-C

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Topic: D.04. Vision

Support: FONDECYT 1110292, 1120570, 1140403

ANR-47 CONICYT

ICM-P09-022-F

Title: Role of connexin channels in retinal synaptic transmission in a diurnal rodent

Authors: *A. V. PALACIOS[1], A. PALACIOS-MUNOZ[1], A. VIELMA[1], J. ARAYA[1], A. ASTUDILLO[2], G. VALDIVIA[1], J. HURTADO[1], O. SCHMACHTENBERG[1], A. D. MARTINEZ[1], M. ESCOBAR[2];
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Abstract: Several studies of nocturnal rodents like the rat have shown that connexin channels, namely gap junction and hemichannels, are expressed in most cells of the retina, playing a central role in visual coding. However, little is known about the contribution of these channels to signal processing in the retina of diurnal animals. Here we studied the distribution of connexin channels and their contribution to retinal light responses in the retina of the diurnal rodent Octodon degus (degu) compared to the rat. Cx36 and Cx45 were found to localize mainly to the outer and inner plexiform layers of degu retina, with subtle differences compared to rat, while Cx43 was expressed mostly in cells of the retinal pigment epithelium. Under scotopic adaptation, the connexin channel blocker 18-β-glycyrrhetinic acid (β-GA) induced a significant reduction of the in-vivo ERG b-wave amplitude: -45.1% in degu and -52.2% in rat. However, under photopic adaptation, connexin channel blockage with β-GA strongly increased the b-wave amplitude in degu (+107.2%) while reducing it in rats (-62.3%). These results were confirmed in degu by in-vitro μERG using multi-electrode recordings. Moreover, β-GA, associated to a checkerboard stimulus protocol, also decreased spontaneous and evoked action potential firing in ganglion cells and increased the response latency in ON and OFF ganglion cells (e.g. Table). These results suggest that connexin channels exert a modulatory control of retinal signal processing in function of light adaptation and have a critical impact on the retinal neural code.

Cell type and time-to-peak under control and β-GA conditions

Type Ganglion Cell	Control	β-GA 50μm	Control time-to-peak (ms)	β-GA 50μm time-to-peak (ms)

ON	86	46	64.15 ± 17.14	91.67 ± 52.03
OFF	294	154	47.41 ± 10.73	68.09 ± 33.16
Total	380	200		

Disclosures: **A.V. Palacios:** None. **J. Araya:** None. **A. Palacios-Munoz:** None. **A. Vielma:** None. **O. Schmachtenberg:** None. **G. Valdivia:** None. **A.D. Martinez:** None. **J. Hurtado:** None. **M. Escobar:** None. **A. Astudillo:** None.

Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: NSF Grant 1256782

Title: A nitric oxide-dependent increase in cytosolic calcium enhances spontaneous and evoked GABAergic synaptic transmission in retinal amacrine cells

Authors: ***J. W. MADDOX**, E. GLEASON;
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Abstract: Nitric oxide (NO) synthase is localized to amacrine cell synaptic terminals (Cao and Eldred, 2001) suggesting that NO synthesis is targeted to these synapses. We have previously demonstrated that an NO-dependent increase in spontaneous post synaptic current (sPSC) frequency is dependent upon extracellular Ca^{2+} and independent of soluble guanylate cyclase. Here, we test the hypothesis that NO affects both spontaneous and evoked GABAergic synaptic transmission by increasing cytosolic Ca^{2+}. Cultured chick GABAergic amacrine cells were used for these studies (Gleason et al. 1993). To further examine the effects of NO on sPSCs, amacrine cells contacting other amacrine cells were voltage clamped at -70mV and exposed to the NO donor *S*-Nitroso-*N*-Acetylpenicillamine (SNAP, 500µM). SNAP produced a significant (91%, p=0.008) increase in sPSC frequency. With the Ca^{2+} channel blocker La^{3+} (50µM), the SNAP-dependent increase in sPSCs was eliminated suggesting that the NO-dependent increase of

sPSCs is due to an influx of Ca^{2+}. The $GABA_A$ receptor antagonist bicuculline (10μM) blocked all sPSCs, indicating that NO promotes the release of GABA rather than a different neurotransmitter. To confirm that NO elevates cytosolic Ca^{2+}, cells were loaded with the Ca^{2+} indicator Oregon Green 488 BAPTA-1, AM. SNAP consistently increased cytosolic Ca^{2+}. Co-application of the NO scavenger 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO, 10μM) eliminated the SNAP-dependent Ca^{2+}elevations, suggesting that NO itself is responsible. La^{3+} prevented the NO-dependent Ca^{2+} increase, confirming the extracellular origin of the Ca^{2+}. Cytosolic Ca^{2+} levels remained elevated when NO preceded La^{3+} plus NO, indicating that elevated Ca^{2+} is maintained in the absence of sustained influx and suggesting a contribution from Ca^{2+} stores. To examine the effects of NO on evoked synaptic transmission, isolated amacrine cells were depolarized from -70mV to -20mV for 50ms to evoke Ca^{2+} influx and autaptic currents. SNAP significantly ($p=0.03$) increased evoked PSCs measured as charge transferred at the end of the voltage step. Bicuculline blocked all evoked PSCs in both control and SNAP solutions suggesting that the NO-dependent increase in charge transfer is due to an increase in GABA release, exclusively. These results demonstrate that NO increases the frequency of GABAergic sPSCs by stimulating Ca^{2+} influx and possibly release from stores. NO also enhances evoked GABAergic PSCs, however, the relative contributions of NO-dependent Ca^{2+} influx, voltage-gated Ca^{2+} channel-mediated influx and Ca^{2+} release from stores remains to be elucidated.

Disclosures: J.W. Maddox: None. **E. Gleason:** None.

Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: Taiwan NSF 101-2311-B-002-023

Title: Bilateral intrinsically photosensitive retinal ganglion cells contribute to the symmetric input of the suprachiasmatic nucleus

Authors: *Y.-T. CHANG, C.-C. LEE, S.-K. CHEN;
Dept. of Life Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: In the mammalian retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) are a small subset of retinal ganglion cells that expresses the photopigment melanopsin. These cells convey photic information to several brain regions including the suprachiasmatic nuclei (SCN) for circadian photoentrainment and the olivary pretectal nuclei for pupil light reflex. The SCN, the mammalian master clock, are two nuclei suited above the optic chiasm, and each of them can be divided into two subdivisions, the ventro-lateral SCN (vlSCN) and the dorso-medial SCN (dmSCN). Neurons of vlSCN primarily express vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP), whereas those of dmSCN synthesize arginine vasopressin (AVP). Recent studies have shown that the ipRGCs directly project to the whole SCN through the retinohypothalamic tract (RHT) and this input is bilateral symmetry, unlike 90% contralateral input of the lateral geniculate nucleus (LGN) from regular retinal ganglion cells (RGCs). However, the innervation pattern and the connection map of a single ipRGC in the SCN remain unknown. Here we used a genetic mouse model, a conditional and inducible Cre-LoxP system, to randomly label a single ipRGC in mice and reconstructed their dendritic structure and axonal architecture from retina to brain. We found that the ratio of ipsilateral to contralateral of ipRGCs was approximately one to nine, similar to regular RGCs that innervate the LGN for image forming functions. Strikingly, some of contralateral ipRGCs could bilaterally project to the both ipsilateral and contralateral sides of the SCN, but all of them had similar dendritic morphology regardless of their axonal properties. Furthermore, a single ipRGC preferentially innervated a specific region of the SCN, such as the vlSCN and the dmSCN. Together, these findings indicate that the bilateral ipRGCs increase ipsilateral projections to the SCN and thereby contribute to the about equal input of the SCN. These detail morphological features and innervation patterns in a single cell level provide us greatly basic information of the neural circuitry to understand how light influences the circadian rhythm.

Disclosures: **Y. Chang:** None. **C. Lee:** None. **S. Chen:** None.

Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: NRF Grant 2013R1A1A2059568

Title: Immunocytochemical analysis of intrinsically photosensitive retinal ganglion cells in zebrafish

Authors: ***Y.-J. HUH**[1,2], J.-S. LEE[2], M.-J. JEONG[2], H.-G. KIM[2], C.-J. JEON[1,2];
[1]Biol., Kyungpook Natl. Univ., Daegu, Korea, Republic of; [2]KNU Creative BioResearch Group (BK21), Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: Melanopsin is a photopigment found in photosensitive retinal ganglion cells that are involved in regulation of circadian rhythms and pupillary light reflex. These melanopsin-containing retinal ganglion cells are called intrinsically photosensitive retinal ganglion cells (ipRGCs). The purpose of this study is to identify the ipRGCs in non-mammalian zebrafish (*Danio rerio*), one of the most widely used animal models for biomedical research. The ipRGCs were identified by immunocytochemistry using antibody against melanopsin. A confocal microscopy was used to characterize the morphological classification of the ipRGCs on the basis of somatic positions and dendritic stratifications in the retina. The number of ipRGCs was approximately 1200 cells per retina, and it comprises approximately 1.8% of total ganglion cells. The mean density was 357±37 cells/mm^2. The ipRGCs were classified into four types: M1, M1d, M2, and M3. M1 types had their somata within ganglion cell layer (GCL) and processed sparse dendrites restricted to OFF sublamina (S1) of inner plexiform layer (IPL). The dendritic field size was relatively large compared to other types. M1d types had displaced somata in inner nuclear layer (INL) and resembled M1 types in their dendritic stratification to the IPL. M2 types also had somata in the GCL and their dendritic process to ON sublamina (S6) of the IPL. M3 types had somata within the GCL and processed their dendrites diffusely to middle sublamina of the IPL. The majority of the cells were either M1 or M3 types and M2 types were rarely found. These present results indicate that ipRGCs in the zebrafish contained both similar cell types to the previous studies of various mammalian retinas and unique cell type such as M3.

Disclosures: **Y. Huh:** None. **J. Lee:** None. **M. Jeong:** None. **H. Kim:** None. **C. Jeon:** None.

Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: NSERC 6362-2012 (MP)

NSERC 311892-2010 (JFB)

Title: Comparative analysis of the endocannabinoid system distribution pattern in the retina of mice, tree shrews, and monkeys

Authors: *J. M. BOUSKILA[1,2], P. JAVADI[1], L. ELKRIEF[3], C. CASANOVA[1], J.-F. BOUCHARD[1], M. PTITO[1];
[1]Sch. of Optometry, [2]Biomed. Sciences, Fac. of Med., Univ. of Montreal, Montreal, QC, Canada; [3]Fac. of Sci., McGill Univ., Montreal, QC, Canada

Abstract: The endocannabinoid (eCB) system is comprised of cannabinoid receptors, endogenous cannabinoid ligands (eCBs), and enzymes metabolizing eCBs. This system is widely expressed in the central nervous system, including the retina. The localization of key receptors, particularly CB1 and CB2 receptors (CB1R and CB2R), have been precisely determined in rodent and primate retinas, but the enzymes implicated in the synthesis and degradation of eCBs are not described in detail. We therefore investigated, using immunohistochemistry and confocal microscopy, the localization of the eCB system in the retina of three vertebrates, namely mice, tree shrews, and monkeys. We found that CB1R distribution is highly conserved among these species. In contrast, CB2R expression is variable across these species; in mice, CB2R is found in retinal neurons; in tree shrews, CB2R is found in Müller cell processes of the outer retina and in retinal neurons of the inner retina; in monkeys, CB2R is restricted to Müller cells that span the entire retinal thickness. The most striking result emerging is that CB2R could not be detected in neuronal cells of the primate retina. NAPE-PLD immunoreactivity was restricted to the photoreceptor layer in monkeys. Fatty acid amide hydrolase (FAAH), diacylglycerol lipase (DGL), and monoacylglycerol lipase (MGL) were distributed similarly in these 3 species. These data demonstrate that, while CB1R, FAAH, MGL, and DGL distributions in retina are similar, differences in localization of CB2R and NAPE-PLD were detected among these species. These results provide evidence that this system is different in higher mammals.

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Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: KTEF

Title: Melanopsin, rod and cone pathways independently mediate migraine-related light aversion in mice

Authors: *A. MATYNIA[1], J. KESSLER[2], E. NGUYEN[2], S. PARIKH[2], A. RODRIGUEZ[2], N. BRECHA[2], M. B. GORIN[3];
[1]Ophthalmology, UCLA, LOS ANGELES, CA; [3]Ophthalmology, [2]UCLA, Los Angeles, CA

Abstract: Photoreceptive and nociceptive pathways are physically and mechanistically isolated: the retina contains no known nociceptors and the trigeminal ganglia contain no known photoreceptors. However, experimental evidence hints at alternative mechanisms for light perception in trigeminal circuits: light-enhanced blink reflexes are retained after optic nerve transection and light-induced pain occurs in numerous ophthalmic clinical conditions. Photoallodynia, a painful response to normal light, affects more than 50% of mild traumatic brain injury patients, 80% of migraine patients and many patients with corneal damage, ocular inflammation or cone dysfunction. Preclinical mouse models of photoallodynia, using light aversion as an endophenotype, have the potential to reveal relevant photoreceptive and nociceptive circuits. A customized behavioral assay was used to assess light aversion with a nitroglycerin (NTG)-induced migraine model in combination with genetic or physical lesion of optic pathways in mice: rd1 mice lacking rod and cone photoreceptors, mice lacking melanopsin-expressing cells (OPN4dta) or mice with a 15 second bilateral optic nerve crush. Wild type, OPN4dta and rd1 mice treated with NTG exhibit increased light aversion compared to vehicle controls. The rd1;OPN4dta double mutant exhibits no light aversion, showing that rod/cone and melanopsin pathways represent all photoreceptor input, and are functionally redundant. By contrast, mice one month after a 15 second bilateral optic nerve crush exhibit normal NTG-induced light aversion, indicating either sparing of some optic nerve fibers after severe ONC or the involvement of non-optic nerve pathways. This latter hypothesis is consistent with the recent discovery of melanopsin in the trigeminally-innervated iris and ciliary marginal zone. Expression of proteins in the trigeminal tissue that may mediate light aversion will be examined. These studies establish that light aversion in a preclinical model of migraine uses multiple retinal-brain circuits to elicit light-defensive behaviors, and raise the possibility of a non-optic nerve pathway. These models may help elucidate novel light-pain neurocircuits and provide insights to clinical classification and management of photoallodynia.

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Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: NSC-102-2321-B-002-081

Title: Melanopsin expressing retinal ganglion cells connect to amacrine cells by intra-retinal axon collateral

Authors: *P.-T. YEH, S.-K. CHEN;
Dept. Life Sci., Natl. Taiwan Univ., Taipei City, Taiwan

Abstract: Retinal structure and functional circuit have been study for several decades. It is well known that the information flow of retinal circuit starts form light reception by rods and cones, to horizontal cells, amacrine cells and bipolar cells, and transduces to brain by retinal ganglion cells. However, recent studies indicated that a group of melanopsin containing retinal ganglion cells, intrinsically photosensitive retinal ganglion cells (ipRGCs), send feedback signal to specific sub-population of amacrine cells by unknown mechanism. Intra-retinal axon collaterals of ipRGCs have been reported recently, yet morphology and functions of these collaterals stay unclear. By randomly genetic labeling of ipRGCs in mice, our study shows two morphologically distinct groups of ipRGC intra-retinal axon collaterals. We also found those collaterals connect to amacrine cells. Our finding suggests ipRGCs send backward signal to amacrine cells via intra-retinal collaterals, which may alter retinal functions.

Disclosures: **P. Yeh:** None. **S. Chen:** None.

Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: NIH Grant EY012345

Title: Activity-dependent development of W3 RGC dendritic structure and synaptic function

Authors: *N. TIAN, E. M. ELIAS, P. WANG;
Opthalmology and Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: Recent studies presented contradictory results about the role of synaptic activity on the development of retinal ganglion cell (RGC) dendritic structure and synaptic formation. Increasing evidence suggest that synaptic activity might play different roles on distinct subtypes of RGCs. The aim of this study was to investigate the role of visual experience on the development of dendritic structure and synaptic function of a genetically identified subtype of RGC, the W3 RGCs. We quantitatively examined the development of the dendritic structure of W3 RGCs by imaging the morphology of dendrites as they grow into different layers of the inner plexiform layer (IPL) of the retina during postnatal development. We found that, following initial stratification during the first two postnatal weeks, there is an expansion of dendrites into the outer portion of the IPL during the following 3 postnatal months. This refinement of dendritic stratification is altered by dark-rearing, suggesting that visual input drives circuit refinement of W3 RGCs. To determine the significance of the dendritic refinement on the development of synaptic function, we directly recorded light evoked excitatory and inhibitory synaptic inputs from individual W3 RGCs using whole-cell voltage clamp. Our results showed a significant decrease in both ON and OFF inhibitory currents from P13 to P60. This is inconsistent with the morphological findings, which show changes occurring only in the OFF lamina of the IPL. Finally, we found that the scaling-back of the light evoked inhibitory synaptic currents requires visual inputs. Taken together, these results demonstrate an important role of visual experience on the developmental refinement of dendritic structure and synaptic function of a developmentally plastic RGC subtype.

Disclosures: **N. Tian:** None. **E.M. Elias:** None. **P. Wang:** None.

Poster

725. Retinal Circuitry

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Topic: D.04. Vision

Support: NRF Grant 2013R1A1A2059568

Title: The differential expression of calcium binding proteins in starburst amacrine cells of rabbit retina

Authors: J.-S. LEE[1,2], M.-J. JEONG[2], Y.-N. GU[2], E.-S. LEE[1], *C.-J. JEON[1,2];
[1]Biol., Kyungpook Nat'l Univ., Daegu, Korea, Republic of; [2]KNU Creative BioResearch Group (BK21), Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: Calcium-binding proteins (CBPs) are important components in calcium mediated cellular signal transduction. Among many CBPs, at least three EF-hand CBPs, calbindin D28K (CB), calretinin (CR), and parvalbumin (PV) have been extensively studied in the retina. In the present study, we investigated the expression pattern of these three CBPs in the cholinergic starburst amacrine cells (SACs) that are the most important element for direction selectivity in the rabbit retina. Double-label immunocytochemistry on vibratome sections and single cell injection after immunocytochemistry on whole mounts were carried out in rabbit retinas. We found that all SACs both in the inner nuclear layer (INL) and ganglion cell layer (GCL) contained PV. However, none of the SACs both in the INL and GCL contained either CB or CR in the present study. The results suggest that specifically PV, not CR or CB may act as calcium buffering protein in the SACs in the rabbit retina.

Disclosures: **J. Lee:** None. **M. Jeong:** None. **Y. Gu:** None. **E. Lee:** None. **C. Jeon:** None.

Poster

725. Retinal Circuitry

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Glaucoma Research Foundation

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Title: RGCs can be molecularly divided into three classes before connectivity has developed

Authors: *K. MARTINEZ, A. NISTORICA, N. SWEENEY, D. FELDHEIM;
UCSC, Santa Cruz, CA

Abstract: Retinal ganglion cells (RGCs) convey light information from the eye to higher visual areas. There are ~20 functionally defined RGC types in mice, each of which has a preferred stimulus and is important for certain behaviors. While the mechanisms underlying RGC fate commitment are relatively well understood, little is known about when or how each RGC type develops, in part because of a lack of type-specific genetic markers. We have identified several transcription factors (TFs) that are expressed in RGC subsets. They show minimal overlap and near-complete coverage of RGCs during development and in the adult mouse. Using birthdating experiments at five time points (E12, E14, E16, P0, P10; n= 3-4 subjects), we found that these transcription factors are not expressed in proliferating cells. Immunohistochemical staining revealed that TF segregation occurs at early time points and persists in the adult. Therefore, RGC type identities may be genetically programmed by TFs acting downstream of initial RGC fate choice.

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Poster

726. Visual Motion

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Topic: D.04. Vision

Support: EU FP7 ERC Advanced Investigators Grant 269853

Title: Revealing columnar-level neural correlates of perceptual switches in area hMT using fMRI at 7 Tesla

Authors: ***R. GOEBEL**[1], A. T. VU[2], V. G. KEMPER[1], M. CASTELO-BRANCO[3], K. UGURBIL[2], F. DE MARTINO[1], E. YACOUB[2];
[1]Cognitive Neurosci., Fac. of Psychology and Neurosci., Maastricht, Netherlands; [2]Ctr. for Magnetic Resonance Res., Univ. of Minnesota Med. Sch., Minneapolis, MN; [3]Visual Neurosci. Laboratory, IBILI, Fac. of Med., Coimbra, Portugal

Abstract: In a previous study using 3 Tesla fMRI [1], we have described that mean activity BOLD levels in the human motion complex (hMT+/V5) reflect global motion perception. Stimuli were two overlapping moving gratings (plaids) that can be perceived either as two independently moving, transparent surfaces moving to the left and right, respectively, or as a single surface moving in an intermediate (vertical) direction. We found that area hMT+/V5 is

involved in mediating the switches between the two percepts: BOLD responses increased during perception of two moving surfaces (component motion) and decreased during perception of a single moving surface (pattern motion). In the present 7 Tesla study (n = 5), we asked whether it would be possible to directly relate mesoscopic (columnar-level) direction-of-motion feature representations within area hMT to the perceived interpretation of the plaid stimulus. Functional responses were measured with 3D GRASE (TR=2000, TE = 32 ms; matrix = 256 x 32; 14 slices; 0.8mm iso-voxel resolution) except for localisation of hMT using a GE BOLD sequence (TR=2000ms, TE=18ms, Flip Angle = 60 deg; matrix 110 x 110; GRAPPA = 2; 38 slices; 1.3mm iso-voxel resolution). Data were high-pass filtered and analyzed in original measurement space without resampling (beyond motion correction) and without spatial smoothing. T1 and proton density weighted anatomical data were acquired for segmentation and sampling along cortical sheets at 3 relative cortical depth levels [2]. In the first experiment, we mapped four axes-of motion columns following our previous work [3]. Second, we induced periods of component and pattern motion by slightly changing the luminance at intersections of the bars of the two moving gratings. Finally an ambiguous plaid stimulus was presented where participants indicated by button presses whether they perceived two surfaces moving horizontally or one surface moving vertically. Both induced (experiment 2) and spontaneous (experiment 3) switches between component and pattern motion perception resulted in similar changes in topographic maps (experiment 1) in area hMT. More specifically, perception of component motion was related to enhanced responses in horizontal axis-of-motion columns while pattern motion perception was related to enhanced responses in vertical axis-of-motion columns. These results indicate that not only the transitory phases during perceptual switches but also the content of perception can be read-out directly from the activity patterns across axes-of-motion columns in area hMT. **References** [1] Castelo-Branco et al. (2002) PNAS [2] De Martino et al. (2013) PLoS One [3] Zimmermann et al. (2011) PLoS One

Disclosures: **R. Goebel:** None. **A.T. Vu:** None. **V.G. Kemper:** None. **K. Ugurbil:** None. **F. De Martino:** None. **E. Yacoub:** None. **M. Castelo-Branco:** None.

Poster

726. Visual Motion

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Support: Deutsche Forschungsgemeinschaft - German-Israeli Project Cooperation (DIP 9, JA 945/3-1, SL 185/1-1)

SFB-874 (Jancke/Eysel, INST 213/691-1)

Title: Propagating wave of activity in V1 induced by simultaneous counterchange of luminance at adjacent stimulus locations

Authors: *S. REKAUZKE[1], N. NORTMANN[1], G. ZURAWEL[2], I. AYZENSHTAT[2], H. SLOVIN[2], D. JANCKE[1];
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Abstract: Various neurophysiological studies suggest that neuronal processing of stimulus darkening dominates that of brightening in primary visual cortex (V1). Particularly differences in response onsets were observed, in that neuronal responses to negative contrast increased at faster rates than to positive contrast, in both monkeys and cats. Correspondingly in monkeys, output layer 2/3 neurons tuned to darkening outnumber those preferring luminance increments. Recently, we used voltage-sensitive dye imaging (VSDI) to measure population responses in monkey V1, and showed marked differences between the retinotopic cortical representations of black and white squares surfaces presented separately. The VSD signal reports the sum of membrane potential changes from neuronal populations and is largely influenced by synaptic potentials in the upper cortical layers. Here we exploited the high spatial and temporal resolutions of VSDI to investigate the cortical response dynamics evoked by two neighboring stimuli that changed contrast simultaneously, but in opposite directions. Squares (size=1.5-2.0 deg, with 3-6 deg center to center distance) were presented to the anaesthetized cat on a dark uniform background (2 cd/m^2) and were turned either brighter (from 15 to 95 cd/m^2) or darker (from 95 to 15 cd/m^2). Additionally, single squares each changing dark or bright were presented at both locations. Similar to previous findings in monkey V1, for single squares we found spatial activity patterns that remained local as expected from retinotopic population mapping. Additionally, we found faster rising times when a single square turned dark as opposed to its brightening. Finally, when two adjacent squares switched luminance concurrently in opposite directions, we detected a propagating wave-front of activity originating at the cortical location representing the darkened square and expanding at a speed of approximately ~0.2 mm/s towards the region representing stimulus brightening. Thus, simultaneous stimulus changes led to sequential - wave-like - activation across cortical retinotopy. We suggest its role in the generation of motion signals in V1 based on simultaneous counterchange of luminance contrast at shifting object borders.

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Poster

726. Visual Motion

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Title: Perception and smooth pursuit of vertical acceleration and deceleration

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Abstract: The relationship between perceiving and visually tracking speed changes in the vertical plane is not well understood. The data available suggest that we may have a predisposition to perceive acceleration better in downward motion and an overall advantage for downward pursuit. In the natural environment we are more likely to see downward acceleration and upward deceleration due to the effects of gravity, and such expectations may affect how we detect and visually track acceleration and deceleration. Nevertheless, little attention has been paid to how we perceive vertical deceleration. In this study we manipulated vertical acceleration and the size of the vertical visual field in order investigate the relationship between perception and pursuit of accelerating and decelerating dots moving continuously in the fronto-parallel plane. We manipulated visual field size with the expectation that smaller fields should restrict pursuit (and potentially increase detection thresholds), whereas large ones should encourage pursuit (and decrease detection thresholds). We conducted two experiments. The first used a two-interval two-alternative forced-choice task to measure acceleration and deceleration

detection thresholds. The second used a passive viewing task in which we measured the gain of vertical smooth pursuit eye movements. Gain was calculated in terms of the root mean squared deviations (RMS) of the right eye's position relative to stimulus position during uninterrupted pursuit. The psychophysical data indicated that performance is better for downward motion than for upward motion, and that it also improves as the field size increases. Furthermore, observers detect acceleration and deceleration similarly, regardless of field size or direction. The eye movement data also showed that pursuit is better for downward than upward motion and for large than small fields. However, pursuit appears to be worse for deceleration than for acceleration, and the RMS difference between small and large fields is greater for deceleration than for acceleration. We conclude that the similar effects of field size on speed change detection thresholds and pursuit support the view that pursuit plays a role in the detection of acceleration and deceleration. Moreover, the asymmetry favouring downward acceleration and deceleration detection and pursuit may benefit the observer in terms of navigation and reaching for objects. Although pursuit seems to be more sensitive to acceleration than to deceleration, it is unclear why some asymmetries in pursuit do not manifest themselves in perception.

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DFG grant STE 1430/6-1

Title: Using MVPA to probe the role of V1 in pattern motion processing

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Abstract: Two superimposed drifting gratings can be perceived as two overlapping gratings moving in different directions, or as a coherent pattern moving in a single direction. Whilst the motion direction of component gratings is already represented in visual area V1, the majority of previous studies have found pattern motion processing only from V2 onwards. Here, we question these findings using multi-voxel pattern analysis (MVPA) in two fMRI experiments. In experiment 1, we presented superimposed sinusoidal gratings with varying angles between the two component motions (expressed as α), which were perceived as patterns moving in two different directions. Participants performed a fixation task and a speed discrimination task. Eye tracking was performed to ensure proper fixation. Polar angle retinotopic mapping and a functional hMT+/V5 localiser were used to define regions of interest (ROIs). A classifier was trained to discriminate the two pattern directions. We could decode the two pattern directions significantly above chance in all ROIs, including V1. Then, cross-classification was performed between stimulus pairs with different α. Again, decoding accuracies were significantly above chance, and did not differ significantly between any of the cross-classifications in any of the ROIs. This suggests the classifier did not use component motion signals, but pattern motion information. This conclusion was verified by experiment 2, where we manipulated the perception of square wave gratings to yield either pattern or component motion perception. While a classifier could again generalize across stimuli composed of different component motions when they were perceived as a single moving pattern, its performance dropped substantially in the case where components were perceived. This effect occurred in all ROIs, and shows that our classifier could not have used component motion information to generalise across α in experiment 1, since there we did not observe a significant drop in decoding accuracy with such cross-classifications. Our results indicate that pattern motion direction information is present in V1. Future studies will determine whether this information is due to feedback from higher areas such as hMT+/V5.

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Poster

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Topic: D.04. Vision

Title: Response properties of individual visual cortex neurons to different types of commonly used motion stimuli

Authors: *W. DANG, P. MAIRE, H. M. PETRY;
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Abstract: The objective of this study was to assess how neurons in the visual cortex code information about visual motion. Neuroscientists have used a variety of visual stimuli to elicit responses from motion-encoding neurons. These have included flashing spots, drifting gratings and populations of coherently moving dots (i.e., random dot kinematograms or RDKs). Unfortunately, these types of motion stimuli are not directly comparable as they each contain different visual information (e.g., pure temporal modulation (flashing spots), form and orientation (gratings), speed (gratings and RDKs). Thus, such stimuli may be preferentially analyzed in different stages early in the primary visual cortex by neurons with distinct receptive field spatial-temporal structure. Tree shrews (Tupaia belangeri) are small, diurnal, squirrel-like mammals with a close taxonomic relationship to primates. They provide a good model for study because their quick and agile navigation requires good motion vision. Extracellular recordings were made from neurons in the striate cortex of anesthetized tree shrews in response to computer generated visual motion stimuli. (All procedures were approved by the UofL IACUC.) Responses (action potentials) from 63 single neurons were acquired using a Cambridge Electronic Design i/o interface and analyzed using Spike2 software. 97% of the neurons recorded had receptive fields in the binocular visual field (i.e., central 45deg). The primary goal for each neuron encountered was to assess its response to pure temporal luminance modulation (temporally-modulated flashing spots), to the speed and direction of oriented motion (drifting sine-wave gratings) and to speed and direction of un-oriented motion (RDKs). For the flash and grating stimuli, spike trains were analyzed into Fourier components at each of the stimulus frequencies, and the first harmonic component was used as the response index. For the RDKs, the average firing rate was used as the response index. Data were analyzed with respect to receptive filed location in visual space, and laminar location in striate cortex (by post-hoc histological verification of recording sites). Across cortical layers, 60.3% of neurons preferred flash stimuli and 33.3% responded best to gratings (mostly in layer IV). Transient neurons showed higher peak frequency but not higher cut-off, and a subpopulation of those followed much faster temporal rates of flashes compared to gratings. Neurons that preferred RDKs were rare (6.4%). These results show that the characterization of temporal response properties of cortical neurons is heavily determined by the choice of motion stimulus used to study them.

Disclosures: **W. Dang:** None. **P. Maire:** None. **H.M. Petry:** None.

Poster

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Title: Neural correlates of complex motion signals in larval zebrafish

Authors: *J. E. FITZGERALD[1], R. PORTUGUES[2,3], F. ENGERT[2];
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Abstract: As an animal moves through its environment, a variety of spatiotemporal correlations amongst photoreceptor signals reflect this motion. Classical motion estimation models suppose that animals compute motion from pairwise correlations, but it has recently been shown that humans, flies, and several other animals also utilize higher-order spatiotemporal correlations that signify motion in natural environments. However, how brains represent the complete set of spatiotemporal correlations that animals use to estimate motion remains largely unknown. Here, we show that larval zebrafish perceive motion from higher-order stimulus correlations with a pattern that closely resembles that previously observed in flies. We were thus able to capitalize on the larvae's transparency to image stimulus-evoked and motor-associated calcium signals from individual neurons throughout the entire brain. These experiments revealed that neural responses were distributed across many sensory and motor areas of the brain. The response profiles of individual neurons were highly heterogeneous, with some neurons selectively responding to a single visual stimulus and others responding with intricate patterns. Our statistical analysis thus emphasizes the distribution of response types across and within brain regions. We anticipate that this rich dataset will provide important insights into the algorithms and mechanisms that brains use to compute complex visual signatures of naturalistic motion and generate behaviors.

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Poster

726. Visual Motion

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Program#/Poster#: 726.07/II2

Topic: D.04. Vision

Title: Neuronal basis for an appropriate behavioral response to unapparent wide-field motion in *Drosophila* melanogaster

Authors: *Y. SUZUKI[1,2], T. AONISHI[1], Y. SEKI[3], H. MIYAKAWA[3], T. MORIMOTO[3]; [1]Tokyo Inst. of Technol., Yokohama, Japan; [2]JSPS Res. Fellow, Tokyo, Japan; [3]Tokyo Univ. of Pharm. and Life Sci., School of Life Science, Japan

Abstract: Robust behavioral control in accordance with a certa*in situ*ation is important for the survival of most animals. Can the insect with a tiny brain also robustly recognize and respond to the meaningful information embedded in the noisy stimulus? To investigate the robustness of the insect's behavioral reaction and its underlying neural basis, we examined the wide-field motion perception of unapparent stimuli with respect to both behavioral and neural activity in *Drosophila* melanogaster. We measured the head yaw optomotor response and horizontal systems (HS) cell activity with *in vivo* whole-cell patch clamp recordings. We found that flies have the robust motion discriminative capacity, witch is relatively unaffected by noise, while sensitivity to the stimulus is proportionally reduced with increase of noise. The activity of HS cells is strongly correlated with the optomotor response and also showed the two distinct features. Furthermore, we propose the possible neural mechanisms underlying these features of the HS activity by simulation studies. Our results suggest that flies can perform the appropriate optomotor reaction against unapparent motion information in order to correctly control their flight or walking orientation, which originate from HS cells activities.

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Poster

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Topic: D.04. Vision

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Title: Adaptive changes of contrast sensitivity in context adaptation vs. point adaptation

Authors: *A. PAWAR, T. D. ALBRIGHT, S. GEPSHTEIN;
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Abstract: Background: Sensitivity of the visual system depends on the history of stimulation, but the nature of adaptive change has been controversial. Previous studies found no change (Barlow, MacLeod & van Meeteren, 1976), gain (Clifford & Wenderoth, 1999), or loss (DeValois, 1977) of sensitivity to the adapting stimulus, or sensitivity changed for stimuli very different from the adapting ones (DeValois, 1977; Krekelberg et al, 2006). The inconsistencies are reconciled when the effects of adaptation are viewed over the entire range of visible spatiotemporal modulations of luminance: the domain of the spatiotemporal contrast sensitivity function (Kelly, 1979). From this global perspective, the local changes of sensitivity add up to a global shift of the contrast sensitivity function (Gepshtein et al, 2013), as predicted by a theory of efficient allocation of receptive fields. Methods: We measured a "slice" of the spatiotemporal contrast sensitivity function at six spatial frequencies (between 0.2 and 8 c/deg) at the same temporal frequency (0.5 Hz) using a direction discrimination task. In "point adaptation," static or dynamic high-contrast adapting stimuli preceded the test stimulus on every trial, and different adapting stimulus was used in different runs. In "context adaptation," one stimulus was presented on every trial. Here, the distribution of stimuli across trials constituted the adapting context, which was different in different runs. Results: In point adaptation, sensitivity across several spatial frequencies was suppressed and the sensitivity function shifted away from the adapter. The suppression of sensitivity was particularly strong at the adapting frequency. In context adaptation, contrast sensitivity at low spatial frequencies was enhanced when measured in the context dominated by high spatial frequencies. Contrary to point adaptation, there was no overall suppression of sensitivity. Conclusions: In point adaptation, the presence of adapter on every trial is an ecologically odd event. The uniformly suppressive effect of point adaptation may be an artifact of experimental method. Here and in Gepshtein, Lesmes, and Albright (2013), the more naturalistic method of context adaptation reveals a more nuanced picture, revealing a pattern of gains and losses of sensitivity expected in a visual system that distributes its limited resources according to stimulus statistics.

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Poster

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Title: Responses of MT/MST neurons elicited by dual-grating stimulus: Differences between areas MT and MST

Authors: *K. MIURA, N. INABA, Y. AOKI, K. KAWANO;
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Abstract: The middle temporal (MT) and medial superior temporal (MST) areas involve neurons that respond to visual motion with directional selectivity and transmit the motion signal to their downstream structures that eventually result in perception and/or visuomotor responses. These two areas constitute successive stages of cortical visual motion processing. To understand visual motion analyses along this hierarchical pathway, we studied the activities of directionally selective neurons in MT and MST areas of two rhesus monkeys during ocular following responses to moving large-field grating patterns. Previous behavioral studies in human and non-human primates demonstrated that unique characteristics of short-latency ocular following responses to moving visual stimuli consisted of multiple motion components could be explained by a competitive mechanism, by which salient features in the motion stimulus were extracted. In the present study, to understand the extraction and representation of the features in the motion stimulus in MT and MST areas, we recorded single unit activities during presenting dual-grating stimuli that consisted of two sinusoidal gratings with different spatial frequencies in the ratio of 3:5 (3f and 5f) that generates a periodic pattern with frequency of f. When successive quarter-wavelength step shifts were applied to this pattern, the embedded components shifted one-fourth of their wavelengths in opposite directions (5f, forward; 3f, backward). The contrast ratio was manipulated between the 3f and 5f components from 1/4 to 4. The initial responses of majority of MT/MST neurons to the dual-grating motion stimuli depended on the contrast ratio between the two components. When one component had much higher contrast than the other, the responses were almost dominated by the component with higher contrast, suggesting that both MT and MST neurons represent motion of the component extracted. On the other hand, a different dependence on spatial frequency was observed in MT and MST neurons when the contrasts of the two components were close. The contrast-ratio (C5f/C3f) at which the two components had equal influences on the neural responses was significantly smaller in area MT than MST, suggesting that higher spatial frequencies had stronger impact on the activities in area MT than MST. This is consistent with our previous finding that MT neurons prefer higher spatial frequency compared with MST neurons. We conclude that the MT and MST neurons code for motion of higher and lower spatial frequency components, respectively and that their responses are depending on the relative contrast among the extracted components in the visual motion stimulus.

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Poster

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Future Systems Healthcare Project of KAIST.

Title: Correlation in temporal switching of bistable perception between static and dynamic visual stimuli

Authors: ***W. CHOI**, S. AN, S.-B. PAIK;
Bio and Brain Eng., KAIST, Daejeon, Korea, Republic of

Abstract: When sensory system receives an ambiguous signal, such as Necker cube, human perception often switches between two interpretations. Even though underlying mechanism of this bistable perception is still elusive, the perceptional switching is thought to be induced by top-down activities of brain (Sterzer & Kleinschmdit 2007, Georgopoulos et al. 2010). The reversal time -switching interval between two states- seems an important factor for understanding this bistable system(A. Borsellino et al. 1972), but the meaning and the mechanism of its variation are not completely understood yet. In this study, we examined the temporal dynamics of spontaneous switching in bistable perception, which may provide insight into how brain makes an interpretation of ambiguous sensory information. Our hypothesis is that there must be a correlation between the reversal times measured from different types of test stimuli, if the switching is induced by a common or similar mechanism. To confirm this idea, we performed human psychophysical experiments, to compare the reversal times in two different types of bistable stimuli - the Necker cube as a static case and the racetrack (Jain Siddharth, 2009) for a dynamic. First we found that the measured reversal time is very consistent in each subject, but varies significantly across subjects. Interestingly, the reversal time for the static (Necker cube) and the dynamic stimulus (racetrack) were highly correlated ($R^2 = 0.4390$, slope = 0.4879, N=6). Next, we developed a simple mathematical model to explain the common dynamics of bistable perception. We designed a double-well energy model using destabilization/

restablization process (Kornmeier et al. 2012), and the model could successfully fit the observed temporal features of bistable switching in both static and dynamic cases. Our result suggests that the bistable switchings in static and dynamic visual stimuli can be described by the same model, thus might be induced by the same top-down mechanism. Reference 1. Sterzer, Proc. Natl. Acad. Sci. U.S.A., 104.1, 323-328 (2007) 2. Crowe, Proc. Natl. Acad. Sci. U.S.A., 107.52, 22677-22681 (2010) 3. Borsellino, Kybernetik, 10.3, 139-144 (1972) 4. Jain, PloS one, 4.2, e4536 (2009) 5. Kornmeier, Front. Hum. Neurosci., 6 (2012)

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Poster

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Title: Two mechanisms are required to explain Ocular-Following Responses (OFRs) in humans to white noise stimuli

Authors: ***B. M. SHELIGA**, C. QUAIA, E. J. FITZGIBBON, B. G. CUMMING;
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Abstract: Using white noise stimuli, velocities of ~40°/s produce maximal OFR responses. If this reflected summation of responses to the Fourier components, this peak should occur at speeds ~1.5-2 times greater. This discrepancy suggests a nonlinear interaction between different spatial frequency (SF) components comprising a noise stimulus. We investigated this issue by recording OFRs to 1D noise in 3 human subjects. For a given speed we compared OFRs to white noise with those to stimuli with certain SF bands removed (central SF values varied from 0.0625 to 4 cpd; Gaussian envelope on log scale), and to stimuli containing only those SF bands. Removing low SF components *reduced* OFR magnitudes, and the SF associated with the greatest reduction matched the SF that produced the maximal response when presented alone. This reduction changed rapidly with SF, compatible with a winner-take-all operation. Removing higher-SF components *increased* OFR magnitudes. As speed increased, this effect was larger and extended to lower SF. This suggests that high SF components suppress the OFR. This may be a consequence of the temporal sampling imposed by the display (150Hz). For high SF filters, the

white noise stimulus contains temporal frequencies that exceed the Nyquist limit, and so these components are essentially flickering. In a separate experiment we show that adding a flickering sine-wave grating reduces OFR responses to moving gratings. This suppression is maximal for flickering SF around 0.5-0.8 cpd, and declines as a Gaussian function of SF. This Gaussian function does not depend much on the SF of the moving grating. This effect of flicker produces a good quantitative account of the contribution of high SF components in moving 1D noise patterns. At higher speeds, more SF components exceed the Nyquist limit, producing more response suppression. Its magnitude grows too, since higher speeds produce flicker in lower SF components, closer to those that produce maximal suppression. Thus, the OFRs to 1D noise stimuli are well described by the combination of two factors: (1) A summation of excitatory drive that reflects the responses to individual Fourier components; (2) A suppression by high SF channels where the temporal sampling of the display leads to flicker. Because this second factor grows with increasing speed, it shifts the OFR speed-tuning curves to white noise stimuli towards lower speeds than expected on the basis of linear summation of spatiotemporal components. These findings have one practical implication: even with high refresh rates (150Hz), the temporal sampling introduced by visual displays has an impact on visual processing. At lower refresh rates this effect is even greater.

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Title: Repetition suppression in macaque superior temporal sulcus (STS) for dynamic visual stimuli depicting hand actions

Authors: *P. KURAVI[1], V. CAGGIANO[2], M. A. GIESE[3], R. VOGELS[1];
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Abstract: An attenuated neuronal response to the repeated presentations of static stimuli (repetition suppression) is a well-known characteristic of many neurons in macaque inferior temporal cortex. Recently, Caggiano et al (2013) showed that the spiking activity of mirror neurons in area F5 did not show repetition suppression when movies of hand actions were repeated. To determine whether the absence of repetition suppression in mirror neurons was due to the brain area (F5 versus temporal cortex) or stimulus characteristics (dynamic stimuli of actions versus static stimuli), we measured the responses of macaque superior temporal sulcus (STS) neurons to repetitions of the same movies as previously used in F5. We measured local field potentials (LFP), multi-unit (MUA) and single unit (SUA) activity in 2 monkeys in the dorsal bank of the rostral STS. Monkey fMRI studies showed that this region is activated by hand actions (Nelissen et al., 2011). Throughout the recordings, the monkeys were performing a fixation task during which either the same action (duration = 1360 msec) was repeated (interstimulus interval: 640 msec) or two different actions were presented sequentially. The actions consisted of grasping an object, touching an object, and mimicking of the grasping action without an object present. All actions were presented under two mirror symmetric viewpoints with the hand starting either in the contra- or ipsilateral lower visual field. Static presentations of the hand or object were also presented in interleaved trials. In each monkey, the SUA to the reaching phase of the action showed repetition suppression when the hand action was initiated in the contra-lateral visual field. The median reduction in raw peak firing rate with repetition was 9% for those conditions and neurons for which the response peaked during reaching (N= 80; $p < 0.05$). No repetition suppression was observed at the later phase of the action for which neurons responded less. The repetition suppression for the contralateral reaching phase was also observed for the MUA, with 93 / 121 condition-neuron combinations showing a smaller peak firing rate to the repeated action ($p = 3.6 * 10^{-10}$). Time-frequency analysis of the LFPs showed a marked reduction in power when repeating the action. This repetition suppression was prominent during the reaching phase and significant in each animal for spectral frequencies above 50 Hz. Adaptation effects at lower frequencies were inconsistent across the monkeys. In conclusion, contrary to F5, repetition suppression is present for dynamic hand actions in rostral STS, suggesting differences between visual and premotor cortex in the adaptation to dynamic hand actions.

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Poster

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UW-Madison Graduate School

Wisconsin Alumni Research Foundation

Title: Dynamic and distributed encoding of transparently moving stimuli in cortical area MT

Authors: *X. HUANG[1], J. XIAO[2];
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Abstract: Segmenting visual scenes into distinct objects and surfaces is fundamental to vision. We investigate how overlapping stimuli moving transparently in slightly different directions are encoded by neurons in the extrastriate area MT. Visual stimuli were two superimposed random-dot patches moving simultaneously in different directions at the same speed within a static aperture. In our main experiment, the two directions were separated by 60°. In a subset of experiments, four direction separations (DS) of 45, 60, 90 and 135° were used. At each DS, we varied the vector-averaged (VA) direction of the bi-directional stimuli to characterize the direction tuning. We recorded from over 180 single units in MT from two macaque monkeys as they performed a simple fixation task or a task to differentiate the bi-directional stimuli from a unidirectional stimulus. We found that different MT neurons showed distinct types of response tuning to the bi-directional stimuli. At 60° DS, the average of the responses elicited by the individual components typically had a single peak, located when the VA direction was at the neuron's preferred direction (PD). The response tuning of 1/2 of the neurons to the bi-directional stimuli roughly followed the response average. Surprisingly, the tuning curves of another 1/3 of the neurons were significantly biased toward the responses elicited by one of the motion components and peaked when one component direction was near the PD. Half of these neurons preferred the motion direction at the clockwise side of the two component directions, while the rest preferred the other component. The response bias toward one component direction tended to be consistent across different DS. Another 1/7 of the neurons showed two separate peaks in response tuning, located when either one of the component directions was near the PD. The response tuning to the bi-directional stimuli can be accounted for by a weighted sum of the responses elicited by the motion components plus a multiplicative interaction term between the component responses. For many neurons, the response weights for the two motion components were significantly different. Interestingly, for neurons showing deviation from response

averaging, their direction tuning evolved over time. During the initial 50-ms response, the tuning curve was symmetric with a single peak. Over another 50 ms, the response tuning was either gradually biased toward one component direction, or split into two peaks. These results suggest that segmentation of stimuli moving transparently in slightly different directions is a dynamic process and information regarding individual stimulus components is distributed across neurons in MT.

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The Hundred Talent Program of the Chinese Academy of Sciences

Title: Single-cell response properties of direction-selective neurons in monkey V2

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Abstract: When tested with moving gratings, about 15% of V2 neurons in macaque monkeys were direction-selective (DS) (Levitt, Kiper & Movshon,1994). These DS neurons are clustered and form direction maps in thick and pale stripes (Lu, Chen, Tanigawa & Roe, 2010). So far, the functional contribution of these DS neurons to visual perception is unclear. Here we studied these neurons with optical imaging and electrophysiological methods in order to address this question. Guided by the direction maps obtained with optical imaging, we recorded 81 neurons in V2 direction domains from 2 anesthetized monkeys. Sixty five percent (53/81) of these neurons were direction selective (DI>0.67). Like MT neurons, V2 DS neurons could be well-activated by both moving gratings and moving random dots (RD). For moving RD stimuli placed in the DS neurons' classical receptive field (CRF), activation in their preferred direction was suppressed by adding additional random dots moving along their non-preferred directions (i.e. suppressed by transparent motion), a property similar to those found in the MT neurons

(Snowden, Treue, Erickson & Andersen 1991). Outside their CRF, most V2 DS neurons also have a strong antagonistic surround. As a result, their responses were largely suppressed when the optimal RD stimuli were expanded to cover their RF surrounds. Interestingly, such suppression could be partly relieved by adding additional random dots moving along the opposite (non-preferred) direction (i.e. a large-field transparent motion stimulus). These results indicate that when their RF surrounds are taking into account, V2 DS neurons prefer transparent motion over coherent motion. This is opposite to these neurons' preference when only their CRFs were stimulated. We hypothesize that the strong suppressive surrounds of V2 DS neurons facilitate the detection of motion contrast signal in the visual field, which may in turn contribute to the identification of motion-defined-shapes.

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Poster

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Title: Motion integration across local fields: An illusion analogous to the barber-pole effect

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Abstract: Visual motion perception relies on integration of ambiguous motion information from multiple local motion fields. A remarkable example of motion integration is barber-pole illusion which can be observed when a moving grating is presented through a rectangular aperture. In our previous study, we further showed that two barber-poles presented side-by-side can be integrated as a single barber-pole, indicating that visual motion presented a multiple local fields can be interested to yield a global percept. In the present study, we developed a novel barber-pole-like stimulus by arranging five moving gratings each of which was presented through a square aperture to examine how the arrangement of local field positions determines the integration from local gratings to a global barber-pole . One local field located in the center and the four other

local fields were on the left, right, upper, and lower side of the center local field. We varied the distance between each local field pairs to characterize the effect of proximity on motion integration. The results showed that motion integration, as fathomed by the magnitude of barber-pole effect, was most robust when the distance of distal of local fields was less than 1°. Moreover, motion integration gradually decreased as the field-to-field increased, a finding that is compatible with the law of proximity. We also found that the ratio between distances of vertical and horizontal fields was the most dominant factor that determined the observed barber-pole effect. The results are compatible with the law of perceptual grouping proposed by Gestalt psychology as nearby objects tend to be integrated to yield a global percept.

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Poster

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Title: Perceptual integration of local motion signals

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Abstract: Motion is crucial for everyday tasks, such as navigation and figure/ground segregation. Motion analysis is generally considered to begin with the extraction of local motion signals. Three kinds of local signals are recognized: Fourier (F) signals (Reichardt, 1961), which correspond to 2-point spatiotemporal correlations; non-Fourier (NF) signals, which consist of spatiotemporal correlations of a feature (e. g. edge or flicker (Chubb & Sperling, 1988) and correspond to 4-point spatiotemporal correlations; and glider (G) signals, which include expansion and contraction and correspond to 3-point spatiotemporal correlations (Hu & Victor, 2010). Studies using synthetic stimuli that isolate each of these kinds of motion and their subtypes have shown that each of them elicit behavioral and neurophysiological responses in a wide range of species, from insects to mammals. However, in naturalistic stimuli, these motion signals occur together rather than in isolation (Nitzany & Victor 2014). Here, using a novel class

of synthetic stimuli, we measure how distinct kinds of local motion signals interact. To generate stimuli that contained two kinds of local motion cues but no further visual structure or cues to motion, we created movies of binary checkerboards that contained a specific level of F motion and a specific level of G motion in the same direction, but were otherwise as random as possible (as formalized by a maximum-entropy criterion). These stimuli (20 X 30 grids of 0.45 x 0.45 deg checks, 10 frames/sec) were presented for 1 sec, and the subject's task was to determine the direction of motion in a two-alternative forced-choice task. Thresholds were determined via standard Weibull-function fits. Pilot results in one subject show a strong interaction between F and G motion: combining subthreshold amounts of F and G motion leads to a suprathreshold percept. This cue combination was approximately quadratic. Moreover, there was a suggestion of light vs. dark asymmetry: F signals interacted more strongly with G signals carried by light regions than with G signals carried by dark regions. In sum, perceptual mechanisms that extract local motion signals combine motion signals of different types, in line with their co-occurrence in naturalistic visual inputs. Extensions of this approach may serve as a bridge between synthetic and natural spatiotemporal visual stimuli.

Disclosures: E.I. Nitzany: None. **J.D. Victor:** None.

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Title: Detecting moving objects based on cue conflict between disparity and motion parallax: Behavior and physiology

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Abstract: Detecting objects that move in the world is fundamental to many behaviors. For a stationary observer, an object that moves in the world (hereafter "dynamic object") produces retinal image motion against a stationary background, which is readily detected by motion-sensitive neurons. When an observer moves, stationary objects in the world also have retinal

motion that depends on location in depth (motion parallax), complicating detection of dynamic objects. If the image motion of a dynamic object differs in direction or timing from the image motion of stationary objects, detecting the dynamic object is easier. How can the brain detect a dynamic object when its retinal motion is similar to that of stationary objects? Binocular disparity and motion parallax are primary visual cues for estimating depth. For stationary objects, the two cues provide consistent depth estimates. When an object moves in the world, the depth estimated from its retinal motion will deviate from the depth estimated from binocular disparity. This conflict between disparity and motion parallax cues can be used to detect dynamic objects. Indeed, humans perceive conflict between these cues as object motion in the world (Rogers and Collett, 1989), and can use this information to detect a dynamic object among stationary objects (Rushton et al, 2007). However, the neural mechanisms underlying this perceptual ability are unknown. We developed a behavioral paradigm in which macaques detect a dynamic object during self-motion. We presented 2-4 objects, one dynamic and the others stationary in the world, while animals were translated by a motion platform and maintained fixation on a world-fixed target. The motion of the dynamic object was gained up or down, such that it simulated a stationary object at a depth that was inconsistent with its disparity. The animal selected the dynamic object by making a saccade. We show that animals can detect dynamic objects based on cue conflict between disparity and motion parallax. Neurons in area MT are selective for depth defined by disparity and motion parallax, with depth-sign preferences that can be matched or mismatched (Nadler et al, 2013). We hypothesized that MT neurons with mismatched preferences for disparity and motion parallax can help detect dynamic objects. While animals performed the task, either the dynamic object or a stationary object was placed in the receptive field of an MT neuron. As hypothesized, we found that some neurons with mismatched depth tuning respond more strongly to a dynamic object than to stationary objects at a variety of depths. We suggest that these MT neurons play a specialized role in detecting dynamic objects during self-motion.

Disclosures: **H.R. Kim:** None. **D.E. Angelaki:** None. **G.C. DeAngelis:** None.

Poster

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Title: A cortex-dependent motion discrimination task in head-fixed mice

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Abstract: Head-fixed behavioral paradigms have been very valuable for behavioral neurophysiology as they allow precise stimulus control and motor readout over a large number of trials with high repeatability. Head-fixed behaviors also facilitate experimental access for manipulation and recording of neuronal activity. Mice are being increasingly used to study the neural mechanisms underlying visual perception as the genetic tools available facilitate visualizing, recording and manipulating specific populations of neurons. Mice can perform several head-fixed visual perceptual discrimination tasks in “go/no-go” configurations. However, 2-alternative forced choice (2AFC) behaviors present several advantages to study perception as they are more robust to changes in arousal and perceptual criterion. Here we developed a head-fixed 2AFC motion discrimination task for mice. We trained mice to self-initiate trials by running on a treadmill. Animals were presented monocularly with random-dot kinetogram stimuli, where a percentage of dots moves coherently in one horizontal direction while the rest moves in 8 possible directions with zero net motion. After stimulus presentation, mice have to report the perceived direction of motion by licking one of two available lick ports. Most mice performed hundreds of trials in each session with high accuracy after 3 weeks of training. Mice made around 90% correct choices for stimuli with high coherences and performed significantly above chance for 16% coherence. Performance was minimally affected by variations in dot life-time, motion speed and dot density. However, accuracy increased with both stimulus duration and size, suggesting that mice integrate motion information in time and space. Reversible inactivation of contra-lateral primary visual cortex (V1) via muscimol injections led to a significant reduction in animal performance, showing that the task requires cortical processing. Our results show that mice can efficiently perform visual 2AFC tasks in head-fixed configurations and can extract global motion from noisy stimuli. The task we have developed presents a powerful tool to investigate the neural basis of direction selectivity and motion perception.

Disclosures: **T.G. Marques:** None. **R.F. Dias:** None. **L. Petreanu:** None.

Poster

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Topic: D.04. Vision

Title: Similarities and differences between human and non-human primate stereomotion processing networks

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Abstract: Motion on a two dimensional frontoparallel plane has been well studied and is known to involve brain region MT, but motion in depth has been studied less intensively. This fMRI study targets the neural correlates of stereomotion in human and non-human primates (macaca mulatta). We presented human and non-human primates multilayered random dot stereogram (RDS) stimuli while fixating a center target. RDSs represented a surface moving in depth or random dots in random depths alternating in a blocked design. Subsequently we used the optic flow to functionally localize motion sensitive regions and a unilateral representation to distinguish MST. In our study we report a network of regions involved in the processing of stereomotion. In human and non-human primates we find two common regions, one within superior temporal sulcus (matching optic flow localized region) and the other in lateral intraparietal sulcus. In human subjects we also find region pV3A activated in stereomotion processing, which is not activated in non-human primates. In conclusion, we report a network of regions involved in the processing of stereomotion in human including pMST, pLIP and pV3A. In non-human primates we find pMST and pLIP to be involved in the processing of stereomotion, but not V3A.

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Charles Hood Foundation

Title: Early color deprivation enhances motion sensitivity in mature primary visual cortex (V1)

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Abstract: Tree shrews (Tupaia) possess dichromatic color vision based on short wavelength sensitive (SWS) and long wavelength sensitive (LWS) cones. Deep red-light-rearing (RLR) selectively deprives the SWS cones, and produces a differential stimulation of color and luminance pathways. Our behavioral studies have shown that even after many months of subsequent housing in normal white light, RLR shrews show poorer color discrimination (Petry & Kelly 1991) coupled with enhanced visual motion sensitivity at high temporal frequencies (Callahan & Petry 1995). Retinal ganglion cells of RLR shrews also are tuned to higher temporal frequencies (Lu & Petry, 2002) indicating an initial retinal locus of this effect. But it is unclear whether this atypical high frequency information is processed and represented in primary visual cortex, where many signals have been shown to correlate with perception. Since temporal tuning properties of neurons are transformed as information is processed along the retino-geniculo-striate pathway of tree shrews (there is a substantial drop in temporal frequency preferences as signals pass from the lateral geniculate nucleus into the cortex, Van Hooser et al, 2013), we asked whether the enhancement of visual motion responses in RLR tree shrews was maintained in V1. RLR shrews were reared from birth to 8-wks of age in deep red light (Kodak 1A tungsten illumination), then housed in normal white light until the imaging. At 62-81 days of age, two-photon imaging of the calcium indicator dye Oregon-Green BAPTA-1 was performed (see Johnson et al., 2010). Cells (in layers II and III of striate cortex) were identified by the experimenter, and the fluorescence of each cell was computed by averaging all pixels within a region of interest of radius 10-12 pixels that was centered on the soma. Stimuli were drifting achromatic sine-wave gratings with spatial frequencies ranging between 0.2-0.4 c/deg, and were presented for 5 seconds, with an inter-stimulus interval of equal duration. V1 neurons of RLR shrews exhibited significantly higher temporal frequency preferences than control animals. This result indicates that striate cortex is capable of processing higher temporal frequencies than those commonly recorded in normally-reared animals. It also suggests that temporal filtering mechanisms in cortex can be mediated by other inputs (e.g., color pathways). Here, a competitive interaction (likely at earlier levels of processing) during post-natal development is indicated. This finding strengthens the evidence for an enhancement of temporal vision (paired with a deficit in color vision) in RLR shrews that can be perceived and used in their behavior.

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Poster

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Title: Functional connectivity dynamic changes with visual neural activity in zebrafish optic tectum

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Abstract: Functional connectivity, measured as correlations of low frequency fluctuations among different brain regions, have been used in revealing important clues of the infra-structure of functional neural networks in human and non-human primates. A typical approach is to treat threshold-gating correlations as edges that link nodes representing different brain regions. Analysis of this correlation map, or network, depicts the small-world property and intrinsic modules of brain functional architecture in general. Here in our current research, we conducted this method on zebrafish optic tectum (TeO) neural networks. *In vivo*, calcium images were acquired by two-photon microscope from nacre larval zebrafish expressing GCaMP5G under the evalv3 promoter. The fish larvae was embedded in low-melting point agarose that sits between two miniature projectors, on which visual stimuli could be delivered. The experiment consisted of two sessions: resting-state session and visual-active session. In the visual-active session, simple geometric figures with different shapes were presented; while in the resting-state session, the screens remained dark. During each session, a single scanning slice covering the TeO was acquired at 0.91Hz for 700 repeats. After detecting region-of-interest (ROI) that showed calcium transients by an automatic procedure, time-series were extracted for each ROI. In average, about 100 ROIs were detected for each individual. A correlation matrix was calculated between ROIs for each fish after filtering ROI raw time-series with a band-pass filter (0.01Hz - 0.08 Hz). With a threshold of r=0.5, maps of functional connectivity were constructed and visualized by BioLayout software. For each ROI, the intensity of neural activity was measured as absolute deviations of calcium transient ($\Delta F/F$). The results indicated the functional connectivity maps demonstrated three categories by their layout: ring-shape, two-cores, and even-distribution. After quantitating the shape of the layout by a circular index, the results showed a positive correlation between the degree of ring-shape-likeness and the mean intensity of neural activity averaging over ROIs for each fish in visual session ($r = 0.728$, $p < 0.001$, $n = 17$), but no significant

correlation was found in resting session (r = 0.2621, p = 0.3095, n = 17). These results may imply the preferences of the functional connectivity dynamics triggered by transient neural activities supports visual processing.

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Title: Transient responses in area MT correlate with speed-change detection performance

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Abstract: Sudden changes in a visual stimulus can elicit positive or negative transients, depending on a neuron's tuning properties. In area MT, the latency of such transients has been shown to correlate closely with reaction time in a feature-change detection paradigm. Since these results relied on positive transients in response to 100% speed increments, we asked whether and to what extent transients in response to both positive and negative speed changes of different, sub- and supra-threshold magnitude can be correlated with behavioral measures of detection performance. We recorded single- and multi-unit responses from 152 MT neurons of two monkeys, using a wide variety of speed increments and decrements. Using signal detection theory and a threshold model based on the population activity of 300 neurons, we show that the amplitude of both positive and negative firing rate transients closely correlates with detection rates, even reproducing a performance asymmetry regarding detection of accelerations vs. decelerations. The population transient is particularly shaped by a response gain that emphasizes transient responses of those neurons which are only sub-optimally tuned to the stimulating speed, while well-tuned neurons respond as could be inferred from their speed-tuning properties.

Moreover, the distribution of transients' latencies follow the same pattern as the distribution of reaction times, and can be well fitted by psychometric functions previously shown to reliably describe the distribution of reaction times as a function of absolute speed difference, including an offset for higher base speeds. Thus, transients elicited by rapid changes in visual motion provide a reliable neuronal correlate regarding both detection rates and reaction times, based on a simple mechanism and a limited and physiologically plausible number of neurons.

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Title: Behavioral genetic investigation of columnar circuits for motion vision in *Drosophila*

Authors: *S.-T. WU, A. NERN, G. M. RUBIN, M. B. REISER;
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Abstract: Many animals rely upon vision to navigate through their environments. The genetic toolkit available in *Drosophila melanogaster* provides exquisite access to the neural circuits that support these visually guided behaviors. We have investigated the behavioral responses of tethered flies walking on an air-supported ball presented with diverse visual stimuli. We have organized these ~150 stimuli, which probe diverse aspects of motion and object vision, into a compact protocol for behavioral genetic silencing experiments. We used recently developed driver lines to silence distinct neuron types within the lamina and medulla (the first and second optic ganglia beneath the retina) and contrast these results with similar experiments in tethered flying flies. We have confirmed that silencing either the achromatic photoreceptors (R1-6) or the feedforward lamina neurons (L1, L2) resulted in losing most behavioral responses to motion stimuli. Intriguingly, silencing the feedback neurons C2 and C3 resulted in enhanced responses to slow motion stimuli and attenuated responses to fast motion stimuli. Moreover, our finding suggest that C2 and C3 inactivation affects the responses to both progressive (front to back) and regressive (back to front) object motion, implying that feedback neurons play an important role in the core circuitry of both the motion and object detection pathways.

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R01-EY11001

Title: Inhibitory stabilization underlies surround suppression in visual motion processing

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Abstract: Surround suppression has been thought to arise from an increase in local GABAergic inhibition. However, recent experimental and theoretical work has suggested that suppression is mediated by a withdrawal of both excitation and inhibition if the cortex is an inhibition-stabilized network (ISN) regime (Ozeki et al. 2009). Recent extensions to the ISN model (Rubin & Miller, unpublished) predict that contrast-dependent changes in surround suppression should occur in any stimulus feature space, so long as connections are spatially localized in that space. Here, we directly tested the role of GABAergic inhibition in surround suppression and spatial suppression, and tested the contrast-dependence of surround suppression in the direction domain represented in MT. We microinjected GABAA and GABAB receptor antagonists to directly reduce the efficacy of GABAergic inhibition, while monitoring neuronal surround suppression via an injectrode. Recordings were obtained in area of MT in monkeys that were trained to perform a psychophysical motion discrimination task. We found that local blockade of GABAergic inhibition had profound effects on psychophysical performance for stimuli within the classical RF. However, there was no consistent effect on psychophysical spatial suppression, which is thought to be a perceptual correlate of neuronal surround suppression (Tadin et al. 2003). Moreover, as in cat V1 (Ozeki et al., 2004), blocking GABA had little effect on neuronal surround suppression. These results are consistent with the prediction of the ISN model, since the surround stimulus still evokes network effects that withdraw excitation under local blockage of inhibition. To study surround suppression in the direction domain, we varied the spread of direction signals within the classical RF of individual MT neurons. Stimuli consisted of dots with 25% moving in the preferred direction of the neuron (the directional center) and 75% in a range

of other directions (the directional surround). Analogously to increasing the stimulus size in the retinotopic domain, we compared the effect of stimulating only the preferred direction to those obtained when the size of the directional surround was increased. By increasing the number of dots moving in specific directions relative to the preferred, we increased the directional surround from 0 to 90 deg. We found that these additional directions decreased neuronal firing for high-contrast stimuli, but not for low-contrast stimuli, as has been reported for the retinotopic domain. Overall, these results suggest that the ISN can provide a unifying explanation for surround suppression in MT.

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Title: The emergence of cohorts of active neurons in random recurrent networks provides the mechanism for acquiring orientation and direction selectivity

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Abstract: We present random heterogeneous recurrent networks of firing rate neurons whose response to the external input is best characterized by activation of different groups of neurons, which we called the cohorts. With the sufficiently strong recurrent inhibition these cohorts of neurons compete for firing and the winning cohort is determined by the structure of the input. The identities of neurons recruited to and dropped from the active cohort changes smoothly with varying input features. We search for the network parameter regime where activation of cohorts is robust yet easily switchable by the external input and has large repertoire of different cohorts. We apply these networks to model the emergence of feature selectivity in visual cortex. We feed these random networks with a set of orientation and direction non-selective harmonic inputs that vary only in their temporal phase, mimicking the response to the moving gratings. The relationship between the phases that carries the information about the orientation of the stimulus also determines which cohort of neurons is activated. As a result the individual neurons acquire

non-monotonic orientation tuning curves which are characterized by high orientation and direction selectivity. This mechanism of emergence for direction selectivity differs from the classical motion detector scheme, which is based on the nonlinear summation of the time-shifted inputs. In our model these two mechanisms coexist in the same network, but can be distinguished because they have different frequency and contrast dependence. In the context of visual input from moving gratings the activations of cohorts convert temporal phase sequence into population code but we argue can be applied to extract and represent various other relevant stimulus features.

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Title: Improved assessment of Optocollic Reflex parameters for measuring visual performance in birds and other animals

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Abstract: Optokinetic Nystagmus (OKN) and Optocollic Reflex (OCR) are reflexive movements of the eye and head, respectively, which combine to provide gaze stabilisation in a wide range of taxa. OKN and OCR comprise: 1) a slow phase, during which the image of the moving stimulus is fixed on the retina by means of a eye/head movement of the same velocity and direction, and 2) a saccadic reset, in which the eye and/or head ‘snap’ back to the starting position in order to recommence the slow phase. Requiring an intact visual pathway, both may be used to check for deficits, such as might arise from injury or neurodegenerative disorders. These reflexive visuomotor movements are also extremely useful for investigating various aspects of perceptual psychophysics as well as the impact of clinical or experimental treatments on visual function. Existing equipment for OKN/OCR assessment in animals is often cumbersome and limited in the range of stimulus parameters that can be assessed. Traditional

OKN/OCR tests are carried out using a cylindrical drum or curtain containing a contrasting patterned visual stimulus, which rotates axially around the subject and subtends a large portion of the field of view. These stimuli can be time consuming to change, for example when spatial acuity and/or contrast sensitivity is to be assessed, making it difficult to determine a threshold during a given trial. We present an updated solution, utilising LCD technology and an automated system for extracting horizontal head angle from video, which offers a significantly increased range of possible stimulus parameters, meaning that experiments previously requiring several separate trials, can now be accomplished in a single trial. Being more accurate and less invasive, this method ultimately requires fewer subjects and shorter experimental session times than traditional methods. We validated this method by comparing the results with previously reported measures of OCR in pigeons (i.e. saccade frequency with respect to stimulus velocity), replicating its characteristic properties. For example, a small increase in the frequency of saccades [i.e. resets] (sac/s) is observed at stimulus velocities between 0°/s-(2.67sac/s) and 3°/s-(3.5sac/s), an almost linear increase in frequency between 5°/s-(5sac/s) and 40°/s-(18.5sac/s), peak frequency of saccades between 50°/s and 70°/s-(21.6sac/s) and falling away steeply thereafter (5 birds x 2 trials). Moreover, the gain of the OCR slow phase can be readily determined. Thus, this approach offers the potential of using reflexive behaviour to quantify more easily and accurately many additional aspects of visual performance in birds as well as other animals.

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Support: Research Leadership Award RL-2012-019

Title: How praying mantids solve the motion correspondence problem

Authors: G. TARAWNEH, L. JONES, C. ROWE, C. RIND, *J. C. READ;
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Abstract: Motion detection requires matching a static region of spatial features across a temporal series of images. This is known as the motion correspondence problem and solving it is a basic visual function in many animals. We present computational models that account for how the praying mantis performs this task, based on psychophysical data. Mantids (Sphodromantis lineola) were placed 70mm in front of a CRT monitor and viewed a full screen random chequerboard stimulus, moving either left or right in a series of steps. The size of the chequer blocks was varied as was the stepping distance. The time between steps was proportional to stepping distance, so the speed of the apparent motion was constant at 60 deg/s. When the stepping distance was small, the stimulus appeared to humans as a smoothly moving pattern and elicited an optomotor response causing mantids to lean in the direction of motion ("motion detected"). When the stepping distance was made larger, however, mantids responded by either peering or remaining still. We fitted psychometric functions to the data and determined the stepping distance Dmax corresponding to a 50% probability of detecting motion for each chequer block size. Our data reveal that, as for humans, mantis Dmax is a linear function of chequerboard block size. However, unlike humans, mantis Dmax is not a constant multiple of block size, ruling out the simplest feature-based explanations. We constructed and simulated variations of Reichardt motion detectors using combinations of spatiotemporal filters with different spatial scales (but all tuned to the speed of the stimulus) and found that detectors combining several filters fit the data more accurately than single-filter detectors even with a smaller number of free parameters. Thus, both human and mantis Dmax can be explained by postulating that motion is extracted by a set of detectors with different spatial scales. We believe these findings are significant because, unlike the human visual system, no multi-scale mechanisms were previously implicated in an insect's visual system.

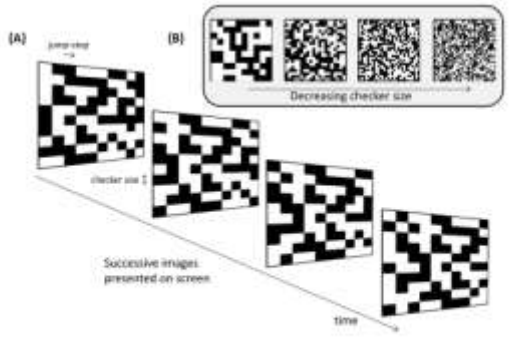

Figure 1: The experimental stimuli. A: Successive images as they appear on-screen. Here, the jump-step is one checker-square to the right. B: Examples of stimuli with different checker sizes.

Disclosures: G. Tarawneh: None. **J.C. Read:** None. **L. Jones:** None. **C. Rowe:** None. **C. Rind:** None.

Poster

726. Visual Motion

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 726.28/II23

Topic: D.04. Vision

Support: National Science Foundation (CRCNS grant IIS-1309725, W.B. and A.P.

Computational Neuroscience Training Grant, 5R90DA033461-03

Title: Modeling adaptive temporal integration in direction selective cortical neurons

Authors: *J. GILE[1], W. BAIR[2];
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Abstract: Direction selective (DS) neurons in the visual cortex are widely believed to underlie the perception of visual motion. The predominant model used to account for the selectivity of DS neurons for spatial frequency, temporal frequency and direction of motion consists of linear filters that are oriented in space-time followed by static nonlinearities, e.g., the motion energy model. The defining features of these oriented-filter energy models, however, do not by themselves capture the results of past studies showing that the window of temporal integration reflected by DS responses changes with the speed of moving stimuli. In particular, in nearly all DS cortical neurons tested, integration time is longer for slower motion and shorter for faster motion. Our goal is to uncover a model that accounts for this phenomenon, known as adaptive temporal integration (ATI), and that also explains the well-established spatio-temporal tuning of DS neurons. Such models will provide better descriptions of the dynamic encoding of local motion signals that drive downstream motion processing and will shed light on the principles of adaptive cortical encoding. We hypothesize that a model based on filters that are curved, i.e., that have a space-time orientation that varies smoothly in time, can account for ATI. To test this, we have developed a scalable system with a Java-based user interface that can apply a variety of optimization strategies to rapidly test different filter parameters (including curves based on sigmoid and power functions) against large amounts of neurophysiological data. Each model is tested with a set of randomly moving sinusoidal grating stimuli at different speeds, a spike-triggered average (STA) is constructed, and metrics of the STA (e.g., width and latency of peak) are computed to compare the output of the model to an existing data set of DS neuronal responses. Our preliminary results show that curved filter models are able to qualitatively capture ATI. We are continuing to test more variations of these models and will compare them to several alternate architectures in their ability to predict a wide range of neurophysiological data. Our long-term goal is to make the resulting models accessible via the web within a cloud-based optimization system to facilitate sharing and further refinement.

Disclosures: **J. Gile:** None. **W. Bair:** None.

Poster

726. Visual Motion

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 726.29/II24

Topic: D.04. Vision

Title: Correlator motion detection for natural stimuli

Authors: ***H. HAFIZI**, S. R. SINHA, R. R. DE RUYTER VAN STEVENINCK;
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Abstract: There have been many studies on correlator-based motion estimation models as the mechanism for biological motion detection and there is experimental evidence in partial support of these models. Here we consider the simplest form of Reichardt correlator model for motion detection and investigate its reliability in the context of natural visual input. To facilitate comparison to a real biological system, we chose model parameters to match the visual system of the blowfly Calliphora vicina. This makes it possible to compare the model results to the behavior of H1, a wide-field motion sensitive cell in the blowfly lobula plate. A dataset of natural stimuli was produced by means of a custom built camera mimicking the hexagonal sampling raster of a small part of the fly's compound eye. The camera was fitted with three gyrosensors which enabled the measurement of yaw, pitch and roll motions. Using this camera outdoors, we recorded light intensities from natural scenes that a fly would see in its natural environment. By design, the light intensities measured by the camera had noise levels much lower than those of real fly photoreceptors. The camera data form the basis of a set of input stimuli for the model. In this study the visual inputs were manipulated in various ways, for example by adding realistic amounts of photon shot noise. In addition we varied the time constant of the correlator. We systematically studied the effects of these manipulations on the bias and the statistical reliability of the model.

Disclosures: **H. Hafizi:** None. **S.R. Sinha:** None. **R.R. de Ruyter van Steveninck:** None.

Poster

726. Visual Motion

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Topic: D.04. Vision

Support: KAKENHI 24330205

KAKENHI 22330198

Title: Velocity selective mechanisms for fast and slow visual motion studied by psychophysics and fMRI

Authors: *I. KURIKI[1], Y. YAMADA[2], K. MATSUMIYA[1], S. SHIOIRI[1];
[1]Res. Inst. of Electrical Communication, [2]Grad. Sch. of Information Sci., Tohoku Univ., Sendai, Japan

Abstract: Human visual system is known to have at least two kinds of visual motion mechanisms that differ in their most sensitive velocity (Shioiri and Matsumiya, 2009). However, the mechanisms of very slow motion, and interactions between the fast- and slow-motion signals are unclear. We addressed these issues with direction selective adaptation technique in both psychophysical and fMRI methods. The subject adapted to a radial gray grating (subtended 2-7 deg in eccentricity; 6 cycles per round), which moved in either clockwise or counter-clockwise direction at either high (4/3 rotation/s) or low (1/18 rotation/s) speed around the fixation point. The adapting stimulus was presented continuously for 12 (+/- 3) s on average, and test stimulus was presented for 3 s; transitions between the stimuli were made gradually with a ramped contrast envelope (0.5 s). This cycle was repeated until collecting 10 trials per condition; the adapting condition (direction and speed) was kept constant during a run. The subjects were asked to report the direction of test stimulus with buttons, as soon as possible. All experiments were conducted in the fMRI scanner to record the behavioral and BOLD-signal responses. Significant motion aftereffects were confirmed for both same speed and different speed pairs of test and adaptation stimulus in perceptual result, measured by the difference of reaction times during the fMRI. The BOLD responses to the test stimuli were analyzed in visual area ROIs localized by retinotopic mapping. In MT+, statistically significant direction-selective aftereffect was found only for the fast test condition after adaptation to the fast motion. In V4, the significant aftereffect was observed for fast and slow test stimuli, only after adaptation to slow motion; V1, V2, and V3 showed the same result with statistical significance. V3AB showed no significant aftereffect in all conditions. We also found the crossed adaptation effect at wide area of the visual cortex, including V1-V3. To investigate the connectivity among visual area ROIs, we also applied covariance structure analysis to the results of fMRI-adaptation experiment. The structure of best-fit model differed with the adaptation condition when tested with the fast-motion

stimulus; a model with a direct path between V1-V3 and V4 and that between V1-V3 and MT+ fitted best for the results after adaptating to slow- and fast-motion stimuli, respectively. The significant aftereffect under slow-adaptation condition in V1-V3 and V4 and the result of the connectivity analysis suggest that slow motions are processed primarily in the ventral stream of the visual system as a pattern motion.

Disclosures: I. Kuriki: None. **Y. Yamada:** None. **K. Matsumiya:** None. **S. Shioiri:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: NIH 2P01NS047399-05

Blaustein Pain Research Fund (JHU)

Neurosurgery Pain Research Institute (JHU)

Title: Differential TTX-sensitivity along C fiber axons of mice and non-human primates

Authors: *A. H. KLEIN[1], T. V. HARTKE[1], R. DE COL[4], R. CARR[4], M. SCHMELTZ[4], J. MANKOWSKI[2], F. BOSMANS[3], M. RINGKAMP[1];
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Abstract: Two types of voltage-gated sodium (Nav) channels are expressed in the soma of primary afferent nociceptive neurons. Tetrodotoxin sensitive (TTX-S) Nav channels such as Nav1.1-Nav1.7 are completely blocked by 100 nM TTX, whereas TTX-resistant (TTX-R) channels such as Nav1.8 and Nav1.9 are unaffected by TTX up to 100 μM. It is currently unclear whether TTX-S and TTX-R channel isoforms are expressed uniformly along the axonal membrane of nociceptors or, alternatively, whether TTX-S and TTX-R channels are expressed in a differential, site specific manner. To test the relative contributions of TTX-S and TTX-R Nav channels to action potential initiation and conduction along the neuronal membrane, we investigated the effects of TTX on C-fiber compound action potentials (C-CAP) of dorsal roots, peripheral nerves, and peripheral distal branches of nerves in C57Bl6 mice and pigtail macaque

(Macaca nemestrina). In the dorsal root of mice and primates, 1 μM TTX reduced the C- CAP amplitude to 6.9±1.1% and 7.1±1.9% of the baseline amplitude, respectively. In de-sheathed peripheral nerves, 1 μM TTX reduced the C-CAP amplitude to 8±1.3% in mice and 13.9±3.1% in primate. In contrast, in de-sheathed distal peripheral branches (i.e. digital nerves) of mice and primates, 31.5±4.8% and 33.4±9.3% of the C-CAP amplitude was resistant to 1 μM TTX, which was significantly different from the CAP amplitude under TTX in dorsal roots and peripheral nerves. After subsequent application of a Nav1.8-specific blocker (A-803467), the C-CAP amplitude was significantly attenuated to 22.9±4.1% in mice and 20.7±8.4% in primates. These data provide direct evidence for a differential expression of TTX-S and TTX-R Nav isoforms along the axonal membrane of C-fibers of mice and primates, and suggest that TTX-R Nav channels are more concentrated in the peripheral distal branches of unmyelinated fibers.

Disclosures: **A.H. Klein:** None. **T.V. Hartke:** None. **R. De Col:** None. **R. Carr:** None. **M. Schmeltz:** None. **J. Mankowski:** None. **F. Bosmans:** None. **M. Ringkamp:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: NIH grant NS042595

NIH grant NS076324

Title: Human sensory neurons: sodium and potassium channel conductances underlying excitability

Authors: ***B. A. COPITS**[1], S. DAVIDSON[1], J. ZHANG[2], G. PAGE[2], A. GHETTI[2], R. W. GEREAU, IV[1];
[1]Pain Center, Dept of Anesthesiol., Washington Univ. Sch. of Med., St Louis, MO; [2]AnaBios, San Diego, CA

Abstract: Biological differences in sensory processing between human and model organisms may present significant obstacles to translational approaches in treating chronic pain. Such obstacles may include functional differences in target receptor pharmacology and signaling or fundamental differences in neuronal physiology. While cultures of rodent dorsal root ganglion

(rDRG) neurons have proven useful for identifying new analgesic targets and elucidating signaling pathways, notable translational failures have recently raised questions about the wisdom of developing drugs for pain relief in rodents for eventual use in humans. While limited evidence suggests that questions regarding human sensory neuron physiology may be addressed in recordings from cultured human DRG, tissue is often obtained from patients with chronic pain or before the nervous system has fully developed. We have characterized the electrophysiological properties of cultured hDRG from healthy, young adult organ donors using whole-cell patch clamp recordings from over 250 neurons. We established that these cells are stable across a range of days *in vitro* and exhibit action potential waveforms similar to those found in rodent nociceptive neurons. These cells were found to respond to a broad range of algogenic and pruritogenic agents, and become sensitized by inflammatory mediators, further suggesting that we are able to target human nociceptive neurons in these preparations. Our data include the contributions of sodium and potassium currents underlying human DRG excitability in voltage-clamp recordings. Preliminary experiments have demonstrated large amplitude sodium currents during depolarizing steps, with a wide range of responses from TTX-sensitive and resistant channels. We are in the process of determining the contributions of different potassium channel populations using a variety of pre-pulse inactivation steps and pharmacological isolation with tetraethylammonium and 4-aminopyridine. Our results indicate that primary cultures of adult human sensory neurons are viable for understanding the neurobiology of human nociceptor signaling and may serve as an excellent platform for the development of clinical therapeutics.

Disclosures: **B.A. Copits:** None. **S. Davidson:** None. **J. Zhang:** A. Employment/Salary (full or part-time):; AnaBios. **G. Page:** A. Employment/Salary (full or part-time):; AnaBios. **A. Ghetti:** A. Employment/Salary (full or part-time):; AnaBios. **R.W. Gereau:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: DoD W81WH-12-GWIRP-IIRA

Title: Classification and characterization of vascular afferents in the rat

Authors: *B. Y. COOPER[1], T. J. NUTTER[2], V. P. DUGAN[3], R. D. JOHNSON[3];
[2]Oral and Maxillofacial Surgery, [3]Physiological Sci., [1]Univ. Florida, GAINESVILLE, FL

Abstract: Introduction. A series of studies have revealed that current signature classified afferents exhibit tissue specific innervation and distinct expression patterns of protein subunits mediating nicotinic (α_7, $\alpha_3\beta_4$;$\alpha_3\beta_4\alpha_5$), protonergic (ASIC1-3), heat sensing TRP (V1, V2), and 2 pore potassium channels(K2p; TASK-1, 2, 3). In contrast, the type 8 nociceptor could be traced from multiple tissues (hairy skin, glabrous skin, muscle, distal colon, penile mucosa). We have recently shown that type 8 neurons exhibit persistent molecular alterations following an 8 week exposure to insecticides linked to Gulf War Illness (Nutter and Cooper, 2014). We hypothesized that type 8 neurons might also be tissue specific, but represent a class of nociceptors that innervate tissues common to all injection sites. In the present studies we examined whether type 8 could be traced specifically from venous tissues. Methods. Experiments were conducted on young adult male Sprague-Dawley rats (n=6). Several centimeters of the left tail vein were isolated, a luminal plug placed at the cranial end, and the vein sutured closed at the caudal end. Two weeks following the injection of DiI-paste, DRGs were excised and plated on 35 mm Petri dishes. Whole cell patch experiments were conducted on highly fluorescent neurons. Only cases without postmortem evidence of dye leakage outside the vascular compartment were accepted. Following current classification procedures (Petruska et al., 2002), neurons were exposed to ACh (500 μM), capsaicin (1 μM) and pH 6.0solutions.Results. Both known and previously unidentified type 8 neurons were labeled by DiI. Many vascular afferents manifested characteristics of kinetically distinct type 8a and 8b neurons (capsaicin sensitive (CAPS), fast and slow kinetic ASIC responders; n=7). Capsaicin insensitive (CAPI) type 8 afferents (n=7) exhibited significantly higher H-current (4.1 +/-1 vs 1.3 +/-.5 pA/pF; $p<.02$), smaller cell size (59.5 +/- 2.4 vs 78.3 +/- 5.5 pF; $p<.02$) and narrower action potentials (4.7 +/- 0.2 vs 8.1 +/- .6 msec; $p<.0003$). CAPI type 8 neurons responded to pH 6.0 with small, non-desensitizing, K2p - like currents (127 +/-24.2 pA; n=7). Two distinct cholinergic response forms were represented in CAPS neurons: slow desensitizing, $\alpha_3\beta_4\alpha_5$-like currents(n=4), and irreversible holding current shifts suggesting closing of a resting current (156 +/- 52 pA; n=3).Conclusions. Type 8 nociceptors innervate vascular tissue. These include previously identified type 8a and 8b CAPS responders and a new type 8c CAPI subclass. Cholinergic response patterns suggested further specializations among vascular afferents.

Disclosures: B.Y. Cooper: None. **T.J. Nutter:** None. **V.P. Dugan:** None. **R.D. Johnson:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: KAKENHI

Title: Nerve injury increases cAMP sensor Epac1 and Epac2 in the distinct subgroups of DRG neurons

Authors: ***M. MATSUDA**, S. YAMAKITA, Y. YAMAGUCHI, Y. IZUMI, M. SASAKI, F. AMAYA;
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Abstract: Background Exchange protein directly activated by cAMP (Epac) is guanine nucleotide exchange factor for the small GTPases and the target of cAMP. In the primary afferent neurons, Epac participates to the chronicity of pain hypersensitivity. In the present study, we investigated the expressions of Epac1 and Epac2 in the DRG, and investigated its regulation in the spinal nerve ligation (SNL) model rats. Methods Male Sprague-Dawley rats weighing 200-250g were used for the experiments. SNL was performed under deep isoflurane anesthesia. Rats treated with SNL or naive controls were transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in 0.1M PB under deep anesthesia. L4 and L5 DRGs were isolated and processed for immunohistochemistry. Visualization of Epac1 and Epac2 was performed using fluorescent immunohistochemistry with anti-Epac1 and anti-Epac2 antibodies by tyramide signal amplification. Double-labeled fluorescent immunohistochemistry for Epac1 and Epac2 with NF200 was also performed. The amount of Epac1 and Epac2 mRNA in the DRG was determined by quantitative real-time PCR. Results In naive animals, Epac1 and Epac2 were detected in small sized DRG neurons. Satellite ganglion cells in the DRG were also positive for Epac1 and Epac2. In naive condition, Epac1 and Epac2 positive profiles were 21% and 23% respectively. However, in SNL treated animals, Epac1 (42%) and Epac2 (37%) positive profiles significantly increased compared to the controls. SNL also induced an increase in the amount of Epac1 and Epac2 mRNA in the DRG. Double-labeling immunochemistry revealed that Epac1 and Epac2 expression was detected in NF200-negative DRG neurons. Conclusions Expression of Epac1 and Epac2 in small-sized NF200-negative DRG neurons implicates their crucial role for the activation of C-fiber nociceptors. Increased expression of Epac1 and Epac2 in the DRG after the SNL suggests their contribution to the development of pain hypersensitivity after the nerve injury.

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Poster

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PUMC&CAMS/IBMS Dean's Fund #2011RC01 (CM)

PUMC&CAMS Youth Research Grant #201211 (CM)

Title: Neuronal Fc-epsilon receptor I contribute to antigen-specific pruritus in a mouse model of allergic conjunctivitis

Authors: F. LIU[1], H. JIANG[1], X. SHEN[1], Z. CHEN[1], L. XU[1], N. CHEN[1], M. ZHOU[1], C. LI[1], T. WANG[1], B. YUAN[1], Y. XIE[1], *C. MA[1,2];
[1]Dept. of Anatomy, Histology and Embryology, Peking Union Med. Col. & Chinese Acad. of Med. Sci., Beijing, China; [2]Anesthesiol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Previous studies demonstrated that some nociceptive dorsal root ganglion (DRG) neurons express Fc-gamma-Receptor I (FcγRI) and can be directly activated by IgG immune complex (IC), and may contribute to antigen-specific pain and hyperalgesia. In this study we investigated the role of nociceptive neuronal Fc-epsilon-Receptor I (FcεRI) in antigen-specific pruritus in an animal models of allergic conjunctivitis. Oval albumin (OVA) and aluminum hydroxide [Al(OH)3] was used as antigen to establish a mouse model of allergic conjunctivitis. The levels of both antigen-specific IgE and IgG in the blood serum of OVA-sensitized mice were found significantly increased. When challenged with the antigen topically to the eye, sensitized mice, but not the naïve ones, exhibited a dose-dependent increasing in the scratching (but not wiping) behaviors toward the challenged eye. This effect cannot be fully blocked by pre-treatment with mast cell stabilizer or histamine receptor antagonist. Western blot and qRT-PCR showed an up-regulation of FcεRIa protein and mRNA, respectively, in the trigeminal ganglion (TG) of sensitized mice. The antigen-induced scratching behaviors were not observed in mice sensitized with OVA but without Al(OH)3, which showed only an increase in the serum level of IgG but not IgE. Immunohistochemistry revealed that FcεRI was expressed in nociceptive TG neurons (but not satellite glial cells). In acutely dissociated TG neurons, IgE-IC produced an increase in the intracellular calcium level and excitability of nociceptive TG neurons. Our results indicate that FcεRI expressed in peripheral nociceptive neurons may involve in the pruritus induced by antigen-specific immune responses via direct activation by IgE-IC, and may suggest

a novel strategy for anti-pruritic treatment in allergic conjunctivitis as well as other diseases with intractable itch.

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Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: NIH/NIDCR Grant R01DE019796

Title: Tyrosinase-dependent peripheral dopamine production mediates baseline pain sensation

Authors: ***K. ONO**, C. T. VIET, D. DANG, Y. YE, B. L. SCHMIDT;
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Abstract: Pain sensitivity differences are present among different strains of mice. These strains also have varying tyrosinase activity, which controls melanin production that causes pigmentation in the skin and hair. Tyrosinase also produces L-dopa that is converted into dopamine. Dopamine has been detected in the skin of black C57BL/6 (B6) mice, but not albino mice. In this study we hypothesized that peripheral dopamine mediates baseline pain sensitivity in mice. We investigated the effect of local dopamine injection on nociceptive behavioral responses and nociceptive receptor expression levels on primary sensory neurons in black B6 and albino tyrosinase-mutated B6 (B6(Cg)-*Tyr*$^{c\text{-}2J}$). In the hind paw and whisker pad, black B6 showed significantly lower mechanical and higher thermal sensitivities than albino B6. Subcutaneous injection of dopamine produced sustained hyposensitivity to mechanical stimulation and hypersensitivity to thermal stimulation. The same sustained changes in mechanical and thermal sensitivity was seen after injection of L-dopa or D1 agonist SKF38393, but not injection of catecholamines or other dopamine receptor subtype agonists. Conversely, the tyrosinase inhibitor kojic acid and the D1 antagonist SCH23390 reversed the strain differences in mechanical and thermal sensitivities. Injection of dopamine and SKF38393 into the whisker pad also downregulated mRNA expression of the mechano-sensitive receptor Piezo2, and upregulated mRNA expression of the heat-sensitive receptor Trpv1, in the associated trigeminal

ganglia. These results suggest tyrosinase-dependent dopamine production mediates expression levels of nociceptive receptors in sensory neurons via D1 activation.

Disclosures: **K. Ono:** None. **C.T. Viet:** None. **D. Dang:** None. **Y. Ye:** None. **B.L. Schmidt:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: NS042595

NS076324

5T32GM007200-38

Title: Increased non-peptidergic intraepidermal fiber density and an expanded subset of chloroquine-responsive trigeminal neurons in a model of dry skin itch

Authors: ***M. V. VALTCHEVA**, V. K. SAMINENI, J. P. GOLDEN, R. W. GEREAU, IV, S. DAVIDSON;
Pain Ctr. and Dept. of Anesthesiol., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Chronic pruritus is a significant clinical problem, but the underlying neurobiological mechanisms remain unclear. Persistent dry skin-evoked itch can be produced by repeated application of acetone and ether, followed by water (AEW) to the cheek skin of mice. Using this model, we examined dry skin-induced changes in intraepidermal innervation and trigeminal neuron physiology. Immunohistological staining for the pan-neuronal marker βIII-tubulin demonstrated a significant increase in total intraepidermal fiber density that was independent of scratching, as mice prevented from scratching by Elizabethan collars still developed dry skin-induced hyperinnervation. To further characterize the effect of dry skin on subsets of peripheral fibers, we examined Ret-positive and CGRP-positive intraepidermal nerve fibers. AEW treatment induced a significant increase in intraepidermal Ret-positive fibers, but not CGRP-positive fibers. To test whether trigeminal ganglion neurons innervating the cheek exhibited altered excitability after AEW treatment, we examined primary cultures of retrogradely labeled neurons using whole-cell patch clamp electrophysiology. AEW treatment produced no

differences in measures of membrane excitability compared with the water-treated controls. On the other hand, calcium imaging studies demonstrated a significant increase in the number of trigeminal ganglion neurons responsive to the non-histaminergic pruritogen chloroquine. Our data indicate that Ret-positive and chloroquine-sensitive neurons are likely to be important for itch related to dry skin and suggest a role for the glial cell derived neurotrophic factor (GDNF) family of ligands (GFLs) in modulating itch. Current studies are investigating the effects of GFL-Ret signaling on spontaneous and pruritogen-evoked scratching behavior, as well as pruritic receptor expression and physiology.

Disclosures: M.V. Valtcheva: None. **V.K. Samineni:** None. **J.P. Golden:** None. **R.W. Gereau:** None. **S. Davidson:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Program#/Poster#: 727.08/JJ3

Topic: D.08. Pain

Title: Botulinum neurotoxin type A is selective for specific subpopulations of sensory neurons in rat DRG cultures

Authors: *C. RHEAUME, M. S. BROWN, K. R. AOKI, J. FRANCIS, R. S. BROIDE;
Biol. Sci., Allergan Inc., Irvine, CA

Abstract: Botulinum neurotoxin type A (BoNT/A) is clinically approved for the treatment of neuromuscular and autonomic disorders and is also used for the treatment of chronic migraine. Additionally, a growing number of studies have indicated that BoNT/A may be effective in treating various pain syndromes. This has led to the hypothesis that BoNT/A ubiquitously inhibits neurotransmitter release from motor, autonomic and sensory nerve endings. The mechanism of action for BoNT/A at the motor nerve terminal (MNT) has been well established and involves binding of the toxin to a receptor complex, internalization, translocation into the cytosol and finally, cleavage of the vesicular docking protein, SNAP25206 into the truncated form, SNAP25197. This results in attenuation of transmitter release from the MNT and inhibition of motor function. We have previously shown that BoNT/A molecular targets - FGFR3, SV2C and SNAP25206 - are broadly expressed and co-localized in autonomic and sensory nerves throughout the rat, primate and human glabrous skin and urinary bladder, suggesting that these nerve endings are equally susceptible to the inhibitory actions of BoNT/A.

However, the distribution of SNAP25197-immunoreactivity (IR) in nerve endings following toxin treatment of these tissues displays a more restricted pattern of expression, indicating a selective uptake of BoNT/A by peripheral nerve fibers. In order to phenotype the sensory neurons susceptible to the effects of toxin, we examined the distribution of SNAP25197 following BoNT/A treatment in neonatal rat dorsal root ganglion (DRG) cultures. Quantitative immunofluorescence was used to characterize the expression of specific biomarkers within sensory neurons of DRG cultures to identify different neuronal phenotypes. In general, the categories of cultured DRG neurons were representative of the *in vivo* populations found in adult whole DRGs. Following BoNT/A treatment of cultures, SNAP25197-IR was expressed in a subset (10%) of total sensory neurons consisting of each of the represented phenotypes. Cleaved SNAP25 was detected in subsets of the non-peptidergic (P2X3), peptidergic (CGRP) and neurofilament (NF)-positive neurons co-expressing a number of different transient receptor potential (TRP) ion channels (TRPV1, TRPA1 and TRPM8). SNAP25197-IR was also expressed in the entire population of tyrosine hydroxylase (TH)-positive sensory neurons. These results demonstrate that under resting conditions, sensory neurons are selectively susceptible to the effects of BoNT/A; however, the factor(s) that regulate this selective uptake remain to be determined.

Disclosures: **C. Rheaume:** A. Employment/Salary (full or part-time):; Allergan Inc. **M.S. Brown:** A. Employment/Salary (full or part-time):; Allergan Inc. **K.R. Aoki:** A. Employment/Salary (full or part-time):; Allergan Inc. **J. Francis:** A. Employment/Salary (full or part-time):; Allergan Inc. **R.S. Broide:** A. Employment/Salary (full or part-time):; Allergan Inc..

Poster

727. Nociceptors: Anatomical and Physiological Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 727.09/JJ4

Topic: D.08. Pain

Support: The Japan Society for the Promotion of Science (No. 23500587)

The Japan Society for the Promotion of Science (No. 24300193)

Title: Temporal histological changes in rat hind paw skin following cast immobilization

Authors: ***Y. SEKINO**[1,2], J. NAKANO[2], Y. HAMAUE[1], S. CHUGANJI[2], J. SAKAMOTO[2], T. YOSHIMURA[1], T. ORIGUCHI[1], M. OKITA[1];

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Abstract: Joint immobilization by casting in humans and experimental animal models results in nociceptive changes in the immobilized limb, as well as sensitization of the spinal cord and dorsal root ganglion (DRG). Although what triggers the neuronal changes following cast immobilization remains poorly understood, we have previously reported epidermal thinning and an increase in peripheral nerve fibers in the hind paw skin, which may be involved in plastic changes in the central pain pathway. However, the detailed changes in skin innervation by the various afferent fiber types following cast immobilization and the mechanism underlying immobilization-induced hyperinnervation remain unclear. Changes in skin innervation are mediated in part by an increase in nerve growth factor (NGF) produced by epidermal keratinocytes. Additionally, keratinocytes express many proteins, such as nociceptive ion channels, more commonly associated with neuronal function. In this study, we investigated 1) changes in nerve fibers, while distinguishing between myelinated A fibers and unmyelinated C fibers; 2) whether NGF expression increases in the epidermis; and 3) whether the expression of ion channels, particularly transient receptor potential vanilloid 1 (TRPV1) and purinergic receptor $P2X_3$, changes in the epidermis in plantar skin of rat hind paw after 1, 2, and 4 weeks of ankle joint immobilization by casting. In the skin of immobilized rats, both myelinated A fibers and unmyelinated C fibers were increased. NGF, TRPV1, and $P2X_3$ expression levels in the epidermis were also increased. Although, the expression levels of NGF did not show a meaningful change through the immobilization periods, other changes became remarkable, depending on the period of immobilization. The time course of the mechanical and thermal hypersensitivity paralleled the time course of these cutaneous histological changes, suggesting a possible causal relation.

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Poster

727. Nociceptors: Anatomical and Physiological Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 727.10/JJ5

Topic: D.08. Pain

Support: Research Project of Kyorin University

Title: Electrophysiological characteristics of chloroquine-sensitive dorsal root ganglion neurons in rats

Authors: *J. YAGI[1], Y. KOBAYASHI[2], N. HIRAI[1], Y. OHKI[1];
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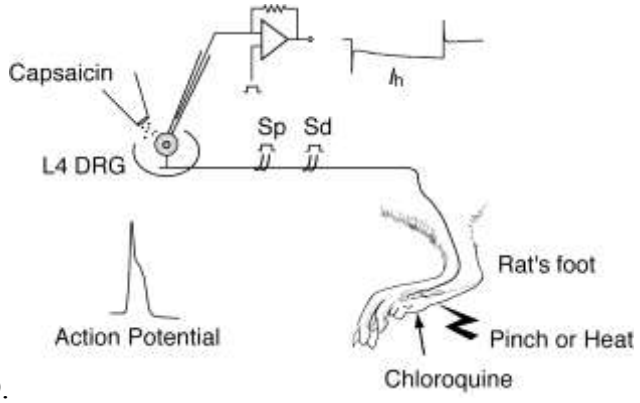
Abstract: Although the sensations of pain and itch are distinct, it has been reported that subsets of nociceptive sensory neurons mediate itch sensation. Here, we used an original method for *in vivo* patch-clamp recording to allow integrated analysis of the diverse properties of dorsal root ganglion (DRG) neurons in rats and investigated the characteristics of chloroqunie (CQ; one of itch-induced chemical agents)-sensitive DRG neurons. Small- and medium-sized DRG neurons that innervate the skin were screened according to axonal conduction velocity (C-type and Aδ-type), action potential duration, current expression profiles (Ih, IA, and T-Ca) and could be classified into 5 classes (Class I-V). Intradermal injection of CQ to the receptive field evoked discharges in some DRG neurons that belonged to Class I characterized by a long action potential and small Ih. They were high-threshold mechanosensitive and also responded to heat or warm stimulation to the receptive field and their somata responded to application of capsaicin. Comparing with the characteristics of CQ-insensitive and nociceptive DRG neurons, we will discuss encoding mechanisms of itch sensation evoked by



CQ.

Disclosures: J. Yagi: None. **Y. Kobayashi:** None. **N. Hirai:** None. **Y. Ohki:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

Location: Halls A-C

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Program#/Poster#: 727.11/JJ6

Topic: D.08. Pain

Support: NIH Grant AI097299

Title: Stress hormone regulation of neuron specificity of herpes simplex virus type 1 and type 2 infection

Authors: *A. IVES[1,2], A. S. BERTKE[2];
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Abstract: Herpes simplex virus types 1 and 2 (HSV1 and HSV2) preferentially establish latent infection in specific subtypes of nociceptors recognized by monoclonal antibody Fe-A5 (Fe-A5+) and the lectin IB4 (IB4+), respectively, from which reactivation occurs to cause different disease manifestations. The viruses also establish latency in autonomic ganglia. HSV-1 reactivates to cause keratitis, cold sores, and rarely encephalitis, while HSV-2 causes recurrent genital lesions and meningitis. Stress hormones corticosterone and epinephrine are involved in initiating the stress response, which is known to reactivate HSV1, but not HSV2, in humans. The mechanism behind stress-induced HSV reactivation has not been investigated at the cellular level in primary neurons. To determine if stress hormones may contribute to different disease symptoms by differentially regulating the behavior of nociceptive and autonomic neurons infected with HSV1 and HSV2, we analyzed co-localization of glucocorticoid (GCR) and adrenergic (AR) receptors with neuronal markers by immunofluorescence in primary adult murine trigeminal (TG) and superior cervical ganglia (SCG) neuronal cultures. GCR was expressed in 39 ± 17.95% IB4+ neurons, 25 ± 6.22% of Fe-A5+ neurons, and 47% of SCG neurons, while AR β2 was expressed in 67 ± 2.6% of IB4+ neurons, 92 ± 3.42% of Fe-A5+ neurons, and 79% of SCG neurons. Thus, GCR and AR β2 are differentially expressed on different types of neurons in which HSV1 and HSV2 establish latency. GCR+ SCG neurons selectively supported productive HSV1 and HSV2 infection but TG neurons limited productive infection, suggesting that corticosterone may have different effects in HSV-infected autonomic compared to sensory neurons. Therefore, corticosterone and epinephrine may regulate autonomic and sensory neurons through different mechanisms to modulate HSV1 and HSV2 infections.

Disclosures: A. Ives: None. **A.S. Bertke:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: NIH DE018661

Title: GTP-dependent run-up of Piezo2-type mechanically activated currents in rat dorsal root ganglion neurons

Authors: Z. JIA, *R. IKEDA, J. LING, J. GU;
Dept. of Anesthesiol. and the Grad. Program in Neurosci., The Univ. of Cincinnati Col. of Med., Cincinnati, OH

Abstract: Rapidly adapting mechanically activated channels (RA) are expressed in primary afferent neurons and identified as Piezo2 ion channels. We made whole-cell voltage-clamp recordings from cultured dorsal root ganglion (DRG) neurons to study RA channel regulation. RA currents showed gradual increases in current amplitude (current "run-up") after establishing whole-cell mode when 0.33 mM GTP or 0.33 mM GTPγS was included in the patch pipette internal solution. RA current run-up was also observed in HEK293 cells that heterologously expressed Piezo2 ion channels. No significant RA current run-up was observed in DRG neurons when GTP was omitted from the patch pipette internal solution, when GTP was replaced with 0.33 mM GDP, or when recordings were made under the perforated patch-clamp recording configuration. Our findings revealed a GTP-dependent up-regulation of the function of piezo2 ion channels in DRG neurons.

Disclosures: Z. Jia: None. **R. Ikeda:** None. **J. Ling:** None. **J. Gu:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: NIH grant DE018661

NIH grant DE023090

Title: Mechanisms underlying the transduction and encoding of tactile stimuli in Merkel discs of mammals

Authors: R. IKEDA, M. CHA, J. LING, Z. JIA, D. COYLE, *J. GU;
Univ. Cincinnati Col. of Med., Cincinnati, OH

Abstract: Sensory systems for detecting tactile stimuli have evolved from touch-sensing nerves in invertebrates to complicated tactile end-organs in mammals. Merkel discs are tactile end-organs consisting of Merkel cells and Aβ-afferent nerve endings, and are localized in fingertips, whisker hair follicles and other touch-sensitive spots. Merkel discs transduce touch into slowly adapting impulses to enable tactile discrimination, but their transduction and encoding mechanisms remain unknown. Using rat whisker hair follicles, we show that Merkel cells are primary sites of tactile transduction, and identify the Piezo2 ion channel as the key Merkel cell mechanical transducer. Piezo2 transduces tactile stimuli into Ca^{2+}-action potentials in Merkel cells, and the Ca^{2+}-action potentials then drive Aβ-afferent nerve endings to fire slowly adapting impulses. We further demonstrate that Piezo2 and Ca^{2+}-action potentials in Merkel cells are required for behavioral tactile responses. Our findings provide insights into how tactile end-organs function and have clinical implications for tactile dysfunctions.

Disclosures: R. Ikeda: None. **J. Gu:** None. **M. Cha:** None. **J. Ling:** None. **Z. Jia:** None. **D. Coyle:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Topic: D.08. Pain

Support: NIH Grant R01DE021996

Title: Gene transfer mediated by sciatic nerve delivery of AAV5-GFP and AAV5-VGF vectors

Authors: *M. S. RIEDL[1], J. A. DYKSTRA[3], K. F. KITTO[1], W.-J. LIN[4], S. SALTON[4], C. A. FAIRBANKS[2], L. VULCHANOVA[1];
[1]Dept Neurosci., [2]Dept Pharmaceutics, Univ. Minnesota, MINNEAPOLIS, MN; [3]Vet and Biomed Sci., Univ. of Minnesota, St. Paul, MN; [4]Dept Neurosci., Icahn Sch. of Med. at Mt Sinai, New York, NY

Abstract: VGF is a granin-related neuropeptide precursor whose peptide products have been implicated in spinal nociceptive signaling and the establishment of chronic pain. VGF expression

is rapidly and robustly upregulated in dorsal root ganglion (DRG) neurons following peripheral nerve injury. To determine whether increased expression of VGF is sufficient for induction of hypersensitivity, we sought to overexpress VGF using a viral vector-mediated approach. Adeno-associated virus serotype 5 (AAV5) vectors have been shown to target dorsal root ganglion neurons following intrathecal injection. However, this route of delivery also results in transduction within several supra-spinal structures, including choroid plexus, hippocampus, and olfactory bulb. Since VGF peptides have been demonstrated to modulate hippocampal neuroplasticity and behavioral measures of depression, evaluation of hypersensitivity following overexpression of intrathecally delivered AAV5-VGF may be confounded by supra-spinal transduction. Based on efficient gene transfer to DRG neurons using intraneural administration of AAV6 vectors, we hypothesized that sciatic nerve injection of AAV5-GFP and AAV5-VGF in rats would result in transgene overexpression that is restricted to DRG neurons. Immunohistochemical analysis of GFP expression revealed low levels of transduction (~ 1%) in DRG neurons, limited labeling in spinal cord, and absence of supra-spinal labeling. Substantial GFP immunoreactivity (-ir) was evident in the sciatic nerve in the area of the injection site, mainly in Schwann cells and perineural connective tissue. AAV5-driven expression of VGF could not be discriminated immunohistochemically from endogenous protein in DRG, however in sciatic nerve the pattern of VGF-ir was similar to GFP-ir. Behavioral analysis included thermal withdrawal latency (Hargreaves method), mechanical withdrawal thresholds (von Frey filaments), and cold allodynia (acetone method). A trend towards mechanical hypersensitivity was noted in AAV5-VGF treated rats with an onset at 2 weeks post-injection and in AAV5-GFP treated rats following 4 weeks after injection. These results indicate that access of AAV5 vectors to sensory neurons following intraneural injection is limited and that transduction of non-neuronal elements in the nerve, including Schwann cells, may contribute to altered mechanical sensitivity.

Disclosures: **M.S. Riedl:** None. **J.A. Dykstra:** None. **K.F. Kitto:** None. **W. Lin:** None. **S. Salton:** None. **C.A. Fairbanks:** None. **L. Vulchanova:** None.

Poster

727. Nociceptors: Anatomical and Physiological Studies

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Program#/Poster#: 727.15/JJ10

Topic: D.09. Tactile/Somatosensory

Support: NIH R25GM097636

NIH NS-065949

Title: Limited incidence of tyrosine hydroxylase-expressing C-fiber low-threshold mechanoreceptors in the first two weeks of postnatal development of mouse

Authors: *R. M. LAM, S. HOCHMAN, M. A. SAWCHUK;
Emory Univ., Atlanta, GA

Abstract: Gentle mechanical stimuli that correlate with the highest pleasantness ratings activate C tactile (CT) afferents in humans (Olausson et al, Nature 5, 2002). The corresponding fibers in mice are C-fiber low-threshold mechanoreceptors (C-LTMRs). One population of C-LTMRs can be identified by selective expression of tyrosine hydroxylase (TH), and these C-LTMRs have the highest incidence of expression in thoracic and sacral spinal dorsal root ganglia (DRG) (Li et al Cell 147, 2011). These afferents are likely recruited during licking, and since maternal licking is important in the development of prosocial behavior, we sought to determine incidence of expression of C-LTMRs in DRGs during development. C-LTMRs were identified by crossing TH-Cre with Cre-dependent tdTomato reporter mice (TH::tdTomato). Mice were perfused at predetermined critical time points during early postnatal development (P7, P10, P14 and P21) and as adults (P33). In the adult, we observed that the incidence and rostrocaudal pattern of tdTomato expression was somewhat lower but comparable to that reported previously with TH immunofluorescence (Li et al 2011). In contrast, there was a much lower incidence of expression in DRGs from mice at postnatal days P21 and earlier. This suggests that C-LTMRs express a mature phenotype rather late in development. Understanding the developmental plasticity of C-LTMRs and the behavioral events associated with their activation should further our understanding of their role in affiliative behaviors.

Disclosures: **R.M. Lam:** None. **S. Hochman:** None. **M.A. Sawchuk:** None.

Poster

728. Thalamic and Cortical Processing

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 728.01/JJ11

Topic: D.08. Pain

Support: Department of Veterans Affairs 1I01BX001629

Title: Chronic pain and thalamic abnormalities following blast-TBI in awake rats

Authors: *A. S. PARIHAR[1], K. KELEDJIAN[2], V. GERZANICH[2], J. M. SIMARD[2], A. KELLER[1];
[1]Anat. and Neurobio., Univ. of Maryland, Baltimore, Baltimore, MD; [2]Neurosurg., Univ. of Maryland, Baltimore, MD

Abstract: Blast traumatic brain injury (blast-TBI) is a leading cause of injury to military personnel. Many suffer from cognitive and motor deficits, as well as from excruciating, unrelenting chronic pain (TBI-Pain). TBI-pain is associated with hypersensitivity to mild tactile and thermal stimulation of the face and scalp, a result of central sensitization, a process by which brain structures undergo maladaptive plasticity, resulting in abnormal activity of brain neurons. We recently demonstrated that after spinal cord injury (SCI), central sensitization occurs in the posterior thalamus (PO). Here, we sought to examine the mechanisms by which blast-induced brain trauma leads to the development of chronic pain. We used a novel model of blast-TBI with two unique features: (i) blast-TBI was performed in awake, unanesthetized rats, to simulate the human experience and to preclude anesthesia-induced dampening of post-injury increases in excitatory activity, that is crucial for the development of central pain; (ii) only the cranium, rather than the entire body, was exposed to a collimated blast wave, with the blast wave striking the posterior cranium in the region of the occipital crest and foramen magnum. Testing for thermal hyperalgesia of the face (distal from direct injury) revealed that blast-TBI rats had a significantly lower tolerance to pain, compared to the control group. Consistent with the behavioral data, single unit electrophysiological recordings from PO showed an increase in the spontaneous firing rate of neurons from blast-TBI rats, compared to sham. These data support the hypothesis that blast-TBI is associated with facial hyperalgesia and maladaptive plasticity in the PO thalamus.

Disclosures: A.S. Parihar: None. **K. Keledjian:** None. **V. Gerzanich:** None. **J.M. Simard:** None. **A. Keller:** None.

Poster

728. Thalamic and Cortical Processing

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Program#/Poster#: 728.02/JJ12

Topic: D.08. Pain

Support: Texas Norman Hackerman Advanced Research Program 003656-0071-2009

NIH 1R01DE022129-01A1

TxMRC

Title: Increase of local field potential in the ventral posterior medial nucleus of the thalamus by activation of chemorhodopsin

Authors: *J. N. STRAND[1], X. ZOU[2], A.-L. LI[1], A. L. HARRIS[1], M. M. KAJUMBA[1], Y. B. PENG[1], L. L. BELLINGER[2], P. KRAMER[2];
[1]Univ. of Texas At Arlington, Arlington, TX; [2]Texas A&M Univ. Baylor Col. of Dent., Dallas, TX

Abstract: Local field potential (LFP) offers a unique look into the summation of the synaptic processes for all cellular activity at a specific neural site. Regarding nociceptive information, LFP can inform on the response of a specific brain area to both noxious and analgesic stimuli. The ventral posterior medial thalamic nuclei (VPM) processes somatosensory information from the head and facial muscles. Thus, the VPM is the ideal location to investigate changes in LFP in response to various stimuli, as in the trigeminal pain model. The VPM of Sprague-Dawley rats were stereotaxically (AP 3.6 mm, midline 3.0 mm, depth 6.4 mm) infused with 250 nanoliters of adeno-associated virus (AAV8) vector (Gene Therapy Center Vector Core, University of North Carolina at Chapel Hill) containing a DNA construct with a modified acetylcholine Gi protein-coupled receptor driven by the synapsin-1 promoter (chemorhodopsin, neuronal specific). Instead of binding to its native ligand acetylcholine, the receptor was modified to bind clozapine-N-oxide (CNO) to induce activation of Gi. Two weeks after virus infusion a ligature was placed bilaterally on the masseter tendon to induce a myogenic orofacial pain, a model for temporomandibular joint (TMJ) disorder. Sham animals received anesthesia but no ligature was placed. Under isoflurane anesthesia, the LFP of VPM was investigated in response to an injection of 50µL of 3% formalin. After establishing a baseline rats in the drug group (containing both ligature and sham rats) received a CNO intraperitoneal injection (1 mg/kg) followed by a formalin injection 1.5 hours later. Rats in the no drug group (both ligature and sham) followed the same procedure but received no CNO treatment. We found (1) The formalin evoked response resulted in a significantly increased LFP ($p< 0.05$) for the ligature/CNO group compared to the ligature/no CNO group Delta, Theta, Alpha and Beta wavelengths. (2) The formalin evoked response resulted in significantly increased LFPs ($p< 0.05$) for the sham/CNO group compared to the sham/no CNO group at all wavelengths. In conclusion, LFP in the VPM was intensified by the activation of Gi in the presence of chemically noxious stimuli. It could potentially contribute to the inhibition of the CNO-activated chemorhodopsin on inhibitory GABAnergic interneurons, which leads to disinhibition of the VPM projection neurons.

Disclosures: J.N. Strand: None. **X. Zou:** None. **A. Li:** None. **A.L. Harris:** None. **M.M. Kajumba:** None. **Y.B. Peng:** None. **L.L. Bellinger:** None. **P. Kramer:** None.

Poster

728. Thalamic and Cortical Processing

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 728.03/JJ13

Topic: D.08. Pain

Support: Texas Norman Hackerman Advanced Research Program (003656-0071-2009)

TxMRC

NIH (1R01DE022129-01A1)

Title: Increased local field potential activity in the anterior cingulate cortex (ACC) in response to inflammatory and mechanical stimulation in freely moving rats

Authors: *A. HARRIS[1], A.-L. LI[2], M. M. KAJUMBA[2], X. YANG[3], J.-C. CHIAO[2], Y. B. PENG*[2];
[1]The Univ. of Texas At Arlington, Arlington, TX; [2]Univ. of Texas at Arlington, Arlington, TX; [3]yangxiaofei@mail.hust.edu.cn, Wuhan, China

Abstract: The Anterior Cingulate Cortex (ACC) is one of the key areas for processing high intensity stimulation that is correlated with pain unpleasantness. Numerous animal studies have implicated activation of the ACC in rodent pain models using methods such as behavioral assays, lesions, imaging, immunohistochemistry, cannulation, and electrophysiology in anesthetized animals (Donahue et al., 2001; Borsook & Becerra, 2009; Yi et al., 2001; Cao et al., 2009; Uhelski et al., 2012; Zhang et al., 2012; Fan et al., 2010; Senapati et al., 2005). However, there is a limitation of tools for detection of brain activity in response to nociception in awake animals. Local field potential (LFP) has recently drawn great attention to achieve this goal. It was hypothesized that spontaneous and mechanically evoked ACC activities would increase during an inflammatory pain model induced by injection of carrageenan into the hind paw. Baseline sensory thresholds were assessed using the mechanical paw withdrawal threshold (MPWT) test in 14 Sprague-Dawley rats. Electrodes were implanted in the right ACC (.7 mm lateral, -3.2 mm dorsal at 15 degrees). After recovery, baseline LFP activity was recorded using our recently developed wireless module. An injection of .05ml of 1% carrageenan or .05ml saline (control) was administered to the intraplantar left hind paw. Four hours were allotted for optimal development of inflammation before the MPWT test was performed again. Then, LFP was recorded at rest and during repeated Von Frey stimulation. Power spectrum analyses were

performed on raw LFP data using CED Spike2 software, as presented in standard frequency bands (Delta 0-4 Hz; Theta 4-8 Hz; Alpha 8-13 Hz; Beta 13-30 Hz; and Gamma 30-100 Hz). Repeated measures ANOVAs were run in SPSS for each frequency band over time (baseline, after injection, after injection with stimulation). Results showed: (1) The carrageenan injection induced tactile allodynia (but not saline) as measured by the MPWT test ($p < .05$). (2) There was a significant increase in LFP activity after carrageenan injection in Delta, Theta, and Alpha frequency bands ($p < .05$). (3) Mechanical stimulation further increased LFP activity during Delta, Theta, Alpha, and Gamma frequency bands ($p < .05$). (4) Saline injection did not evoke significant changes of activities from baseline, even after mechanical stimulation was applied ($p > .05$). These findings suggest that (1) LFP is a valid tool to detect brain activity changes in response to nociceptive input; and (2) it provides electrophysiological evidence in freely moving animals that confirms the role of the ACC during spontaneous and evoked inflammatory pain.

Disclosures: A. Harris: None. **A. Li:** None. **M.M. Kajumba:** None. **X. Yang:** None. **J. Chiao:** None. **Y.B. Peng*:** None.

Poster

728. Thalamic and Cortical Processing

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Program#/Poster#: 728.04/JJ14

Topic: D.08. Pain

Support: Texas Norman Hackerman Advanced Research Program (003656-0071-2009)

TxMRC

NIH (1R01DE022129-01A1)

Title: Local field potentials of the central nucleus of the amygdala (CeA) induced by formalin injection

Authors: *M. KAJUMBA[1], A.-L. LI[1], A. HARRIS[1], X. YANG[2], J.-C. CHIAO[3], Y. B. PENG[1]; [1]Psychology Dept., Univ. of Texas At Arlington, Arlington, TX; [2]Electronic Sci. and Technol. Dept., Huazhong Univ. of Sci. and Technol., Wuhan, China; [3]Electrical Engin. Dept., Univ. of Texas at Arlington, Arlington, TX

Abstract: The recording of the pain related activities in the brain involves use of various electrophysiological techniques. Local field potentials (LFPs) provide a unique set of data that

can be used for long term brain recordings. The amygdala is one of the key structures involved in emotion and pain processing. The purpose of this study is to investigate LFP patterns in the central nucleus of the amygdala (CeA) in response to the formalin-induced nociception in freely moving animals. We hypothesized that the nociception-related changes in the CeA will be indicated by changes in LFPs, with a potentialdifference between the right and left CeA. Sprague-Dawley rats were used in the study; LFPs were recorded either under anesthesia or in freely moving rats. After implantation of bipolar electrodes into either the left or right CeA a week prior to the experiment, LFPs were recorded by a customized wireless module. After recording baseline activity for 10 minutes, 50µl formalin (3.0%) was injected to either the left or right hindpaw, followed by a 45 minutes recording. At the top of the hour the procedure was repeated for a formalin injection into the other hindpaw. Our current results indicate that the right CeA shows a trend of increased activity in response to either the first or second formalin injection administered to either the ipsilateral or contralateral hindpaw. On the other hand, the left CeA shows a trend of increased and decreased activity in response to the first contralateral and ipsilateral formalin injections, respectively. However, the second contralateral and ipsilateral formalin injections induce a trend of decreased and increased activity in the left CeA, respectively. Our findings are consistent with previous studies which found that the right CeA has bilateral receptive fields while the left CeA only has contralateral receptive fields. Therefore, either the first or second formalin injection on either side will activate the right CeA. Similarly, the first contralateral formalin injection induces increased left CeA activity, while an increased response to a second ipsilateral injection might be due to sensitization induced by the initial contralateral formalin injection. Because of lack of direct input ipsilaterally, the left CeA activities are reduced in response to an ipsilateral formalin injection, possibly due to the activation of inhibitory effect of stress, fear, or other emotions, and this suppressed response of the left CeA may last to the second contrateral formalin injection. A differential response pattern of left and right CeA has been identified.

Disclosures: **M. Kajumba:** None. **A. Li:** None. **A. Harris:** None. **X. Yang:** None. **J. Chiao:** None. **Y.B. Peng:** None.

Poster

728. Thalamic and Cortical Processing

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Program#/Poster#: 728.05/JJ15

Topic: D.08. Pain

Support: NIH Grant NS081121

NIH Grant NS038261

Title: 5-HT_{2C}R knockdown in the amygdala inhibits abnormal neuronal activity in neuropathic rats

Authors: *G. JI[1], T. A. GREEN[2], V. NEUGEBAUER[1];
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Abstract: The amygdala plays a key role in the emotional-affective component of pain and pain modulation. Neuroplasticity in the basolateral (BLA) and central (CeA) amygdala correlates positively with pain behaviors. Previous studies from our group and others showed that the CeA serves major amygdala output functions to generate emotional-affective behaviors and modulate nocifensive responses. CeA output can be modulated by the BLA and evidence suggests that serotonin receptor subtype 5-HT_{2C}R in the BLA, but not CeA, contributes critically to anxiogenic behavior and anxiety disorders. Here we tested the hypothesis that 5-HT_{2C}R in the BLA plays a critical role in the central sensitization of amygdala output neurons (CeA neurons) in neuropathic pain. Extracellular single-unit recordings were made from CeA neurons in anesthetized neuropathic rats (SNL, Chung model) and in sham-operated controls. Rats received stereotaxic injections of 5-HT_{2C}R (or control) shRNA viral vector into the BLA to knock-down 5-HT_{2C}R expression. Background activity and evoked responses of CeA neurons were increased in neuropathic rats compared to sham controls. Altered CeA activity included increased irregular spike firing, increased number and duration of bursts, and decreased inter-burst intervals in neuropathic rats compared to sham controls. Local (BLA) knockdown of 5-HT_{2C}R eliminated the abnormally enhanced CeA activity (irregular spike firing, increased burst activity, shorter inter-burst intervals) in neuropathic rats.The data suggest that 5-HT_{2C}R in the BLA contributes to neuropathic pain-related amygdala activity and 5-HT_{2C}R knockdown in the BLA inhibits abnormal activity in the amygdala output region (CeA).

Disclosures: G. Ji: None. **V. Neugebauer:** None. **T.A. Green:** None.

Poster

728. Thalamic and Cortical Processing

Location: Halls A-C

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Title: The local field potential in the medial prefrontal cortex in response to peripheral nerve stimulation

Authors: *A. LI[1], A. HARRIS[1], M. KAJUMBA[1], X. YANG[2], J.-C. CHIAO[1], Y. PENG[1]; [1]Dept. of Psychology, Univ. of Texas at Arlington, Arlington, TX; [2]Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: Nociceptive stimuli can activate peripheral nociceptors to produce nociceptive signals which can be projected to the spinal cord and further up to the thalamus, cortical and subcortical areas through ascending pathways. Nociceptive information can be processed in various brain areas (e.g., somatosensory cortex and anterior cingulate cortex); it can change activities in those brain areas and eventually result in morphological changes following long-term stimulation. The medial prefrontal cortex (mPFC) has been proven to be involved in higher cognitive functions and emotional processing. It receives projections from brain areas that are involved in nociception. In this study, under general anesthesia, both single cell activity of spinal cord dorsal horn (DH) and the local field potential (LFP) of mPFC were investigated in response to peripheral graded electrical stimulation of L5 spinal nerve by a customized wireless module (10Hz, 1ms, 5s, from 0.1v to 5v with 0.5v increment) and formalin injection. We found: (1) The DH neuronal activity was significantly increased by L5 spinal nerve stimulations equal to or higher than 0.5v ($p < 0.05$), intensity-dependently. Significant after-discharge was also observed at high-intensity stimulations from 3.5v to 5v. (2) Electrical stimulation at 1v or higher showed similar DH response to phase I response after formalin injection. (3) LFP was reduced bilaterally in mPFC by L5 spinal nerve stimulation. The inhibition became significant starting at 1v for contralateral and 3v for ipsilateral mPFC. (3) For contralateral mPFC, delta band didn't show significant inhibition; the inhibition for theta band was mainly around lower-intensity stimulations (0.5v-2v), while inhibition for alpha and beta band was around high intensity stimulations; inhibition in Gamma was not as significant as other frequency bands. (4) For ipislateral mPFC, significant inhibition was mainly seen at high voltage stimulations for all frequency bands. In conclusion, LFP in mPFC was inhibited bilaterally by peripheral sensory input induced by electrical stimulation of L5 spinal nerve. These results are consistent with other studies and indicate the contribution of different pattern of mPFC involvement in nociception.

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Poster

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Support: PI-initiated Research grants from Medtronic, Inc. and Zalicus, Inc.

Title: T-type calcium channel modulator Z944 normalizes cortical power, thalamocortical synchrony, and nociceptive behaviors in a rat model of neuropathic pain

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[1]Neurosurg., Rhode Island Hosp., PROVIDENCE, RI; [2]Medtronic, Inc., Minneapolis, MN; [3]Zalicus, Inc., Cambridge, MA; [4]Neurosci., Brown Univ., Providence, RI

Abstract: Long-term neuronal plasticity secondary to chronic pain is a phenomenon based on single-unit physiology in single brain regions, as well as neuroimaging data. To better understand network dynamics related to nociceptive processing, we recorded local field potentials (LFP) in posterior ventrolateral (VPL) thalamus and primary somatosensory (S1) cortex, simultaneously, in anesthetized rats 7 days following chronic constriction injury (CCI). Our data show that, compared to naïve rats, those with CCI manifest significantly increased LFP power in S1 (+139 ± 58 % total power 3-30 Hz), decreased VPL-S1 synchrony (-27% ± 16 % total coherence 3-30 Hz), and attenuated VPL(theta)-S1(gamma-envelope) cross-frequency coupling (-19% ± 7 % total coherence 4-11 Hz). Using a systemically administered T-type calcium channel modulator known to suppress thalamic bursting *in vitro*, Z944, we found partial but significant reversal of LFP power in S1, increased VPL-S1 synchrony, and restored cross-frequency coupling. In addition, Z944 significantly attenuated thermal hyperalgesia and normalized conditioned place preference in awake rats with CCI. Our data suggest that chronic pain leads to long-term changes in thalamocortical dynamics under anesthesia, in support of previous data from our lab in awake rats with CCI (LeBlanc et al. 2014). Moreover, we speculate that abnormal thalamic bursting is a causal contributor to these pain-related changes at the electrophysiological and behavioral levels.

Disclosures: B.W. LeBlanc: 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.; Research

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Poster

728. Thalamic and Cortical Processing

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Topic: D.08. Pain

Support: NRF Grant 2012R1A2A2A02011838

Pioneer Grant 2013008915

Title: Role of reticular thalamic neurons in formalin induced nociception

Authors: *Y. HUH[1], J. CHO[2];
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Abstract: The reticular thalamus (RT) is a thalamic structure which is mutually connected with the cortex and the thalamus. The RT is mainly composed of inhibitory GABAergic neurons that projects to sensory thalamocortical (TC) neurons and is able to regulate TC neurons to switch between tonic and burst firing modes. The two different firing modes of the TC neurons have been implicated to have differential roles in sensory modulation including pain. However, the role of RT in behavioral pain modulation is unclear. In the current study, the role of the RT in modulating formalin induced nociception was investigated by making lesions in the RT and measuring changes in nociceptive responses and recording TC neuronal activities in behaving mice. Results show that specific lesion of the RT altered formalin induced nociceptive responses and that TC neuronal activities also were altered accordingly to the lesion induced behavioral changes. This indicates that the RT actively participates in modulating formalin induced nociception.

Disclosures: Y. Huh: None. **J. Cho:** None.

Poster

728. Thalamic and Cortical Processing

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Topic: D.08. Pain

Support: CNRS contract UPR3212

Université de Strasbourg

NARSAD

Erasmus Mundus Neurotime

Title: Cortical dissociation of neuropathic pain components: Role of the anterior cingulate cortex and of the posterior insula

Authors: *M. BARROT, F. BARTHAS, J. SELLMEIJER, S. HUGEL, I. YALCIN;
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Abstract: Besides chronic stress, chronic pain is among the prevalent determinant for depression. Specific brain regions involved in the pain experience also process mood-related information, and the alterations induced in these regions by sustained pain may alter the processing of affective information, thus resulting in anxiodepressive disorders. Here, we compared the role of the anterior cingulate (ACC) and posterior insular (pIC) cortices in the anxiodepressive, sensory and affective aspects of chronic pain. Chronic neuropathic pain was induced by inserting a cuff around the right common sciatic nerve. Bilateral excitotoxic lesions of the ACC and of the pIC were performed by local injection of ibotenic acid under stereotaxic surgery. Anxiodepressive-related behaviors were evaluated by using novelty suppressed feeding, splash and forced swimming tests. The mechanical threshold of hindpaw withdrawal was determined using von Frey filaments while the aversive component of spontaneous pain was evaluated by using place conditioning. Optogenetic stimulation of the ACC was done in Thy1-ChR2-YFP mice. The lesion of the ACC completely prevents the anxiodepressive consequences of chronic pain and the aversive aspect of spontaneous pain without affecting the sensory mechanical allodynia. Conversely, the aversive component and the anxiodepressive consequences of chronic pain are still present after lesion of the pIC, even though the mechanical allodynia is suppressed. The repeated optogenetic stimulation of the ACC is sufficient to induce

anxiodepressive behaviors in the Thy1-ChR2-YFP mice. Our results show that, at cortical level, the sensory component of chronic pain remains functionally segregated from its affective and anxiodepressive components. Furthermore, the ACC appears as a specific hub for the anxiodepressive consequences observed in chronic pain, thus constituting an important target for divulging the underlying mechanisms.

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Poster

728. Thalamic and Cortical Processing

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Title: *In vivo* neural recording from ventral posterolateral thalamus and primary somatosensory cortex in the pain pathway during electrical stimulation on anterior cingulate cortex

Authors: ***T. KANG**[1], C. IM[2], Y. CHO[1], H. YOO[1], K. KIM[3], S. JUN[1];
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Abstract: The electrical neural stimulation has been known to be an effective treatment for the neuropathic pain. Recently, it was reported that the neuropathy-induced hyperactivity in specific brain areas can be decreased by electrical stimulation. Especially, it was shown that the electrical stimulation on anterior cingulate cortex (ACC) alleviates the representative characteristics associated with the neuropathic pain. The ventral posterolateral nucleus (VPL) of thalamus and the primary somatosensory cortex (S1) are the important brain regions included in the pain-related neural pathway. In this study, we simultaneously recorded the neural activities from both VPL and S1 of anesthetized rats and applied mechanical stimulation mimicking the pain. The mechanical stimulation induced by clip (9.08 g/mm^2) was applied to hind paw of Sprague-Dawley rats (250-300 g). Along with the mechanical stimulation, at the same time, electrical current stimulation was delivered to ACC to see the effect of electrical stimulation on the pain

signals. The parameters of electrical stimulation were 10-50 Hz, 100 μA pulse amplitude, 60 μs pulse duration and biphasic pulses for 20 seconds. Single unit activities and local filed potentials (LFPs) were recorded from VPL and S1. In single unit activities, spike firing rate was increased during mechanical stimulation but decreased by ACC electrical stimulation in both S1 and ACC. Also, Spike synchronization between VPL and S1 was increased while applying the mechanical stimulation but changed by ACC electrical stimulation. In LFPs, spectral power was increased in high gamma band (80-150 Hz) and decreased in low frequency band (1-10 Hz) during mechanical stimulation in both VPL and S1. In summary, the neural activities in S1 and VPL are very similar and both can be distinctive characteristics of neuropathic pain. Also, it is likely that ACC stimulation is feasible for modulating neuropathic pain by decreasing the synchronization between VPL and S1 in the pain pathway.

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Poster

728. Thalamic and Cortical Processing

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Topic: D.08. Pain

Support: NIH Grant NS081121

NIH Grant NS038261

Title: 5-HT2CR knockdown in the amygdala alleviates pain-related behaviors in neuropathic rats

Authors: *V. NEUGEBAUER[1], T. A. GREEN[2], G. JI[1];
[1]Dept Neurosci & Cell Biol, [2]Pharmacol & Toxicol, Univ. Texas Med. Br., Galveston, TX

Abstract: Pain, including neuropathic pain, has a negative affective component and is often associated with anxiety and depression. Previous studies from our group and others established an important role of the amygdala in pain-related emotional-affective behaviors. Evidence suggests that serotonin receptor subtype 5-HT_{2C}R in the basolateral (BLA) but not central (CeA) amygdala contributes critically to anxiogenic behavior and anxiety disorders, and 5-HT_{2C}R has emerged as a relevant target in the treatment of neuropsychiatric disorders. Here we tested the

hypothesis that 5-HT_{2C}R in the BLA plays an important role in neuropathic pain so that local (BLA) knockdown of 5-HT_{2C}R would inhibit pain-related behaviors of neuropathic rats. Sensory and affective pain-related behaviors were tested in neuropathic rats (SNL, Chung model) and in sham-operated controls. Rats were treated with stereotaxic injections of 5-HT_{2C}R (or control) shRNA viral vector into the BLA to knock-down 5-HT_{2C}R expression. Compared to shams, SNL rats showed decreased foot withdrawal thresholds, increased duration of audible and ultrasonic vocalizations, and decreased open-arm choices in the elevated plus maze test reflecting anxiety-like behavior. Compared to control viral vector treated groups, 5-HT_{2C}R knockdown in the BLA inhibited withdrawal reflexes, vocalizations and anxiety-like behaviors in SNL rats. The data suggest that 5-HT_{2C}R in the amygdala (BLA) contributes to neuropathic pain behaviors and 5-HT_{2C}R knockdown in that brain area has beneficial effects in neuropathic rats.

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Poster

728. Thalamic and Cortical Processing

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Topic: D.08. Pain

Support: NIH Grant NS038261

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Title: Group II mGluR modulates synaptic transmission in the rat medial prefrontal cortex in a pain model

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Abstract: Pain-related increase of synaptic inhibition and decreased output of pyramidal neurons in the medial prefrontal cortex (mPFC) have been shown to correlate with impaired cognitive function in a decision-making task in rats (Ji et al 2010 J Neuroscience; Sun and Neugebauer 2011 J Neurophysiology; Ji and Neugebauer 2011 J Neurophysiology). Therefore, restoring mPFC pyramidal output could be a useful strategy to mitigate cognitive impairments in pain. We showed previously that activation of cannabinoid receptor CB1 can enhance mGluR5 function to

increase activity of mPFC pyramidal cells in an arthritis pain model (Ji and Neugebauer, 2014 Eur J Neurosci). Here we focused on another target, group II mGluR2/3, because they are highly expressed in the mPFC and stimulation of these receptors has been reported to have beneficial effects on cognitive functions. However, the contribution of mGluR2/3 to pain-related synaptic transmission in the mPFC remains to be determined. Therefore, we tested the ability of an mGluR2/3 agonist to restore pyramidal cell output in an arthritis pain model. Whole-cell voltage- and current-clamp recordings were made from visually identified pyramidal cells in layer V of the infralimbic mPFC in brain slices from arthritic rats (5-6 h after intraarticular injections of kaolin and carrageenan into one knee). A selective group II mGluR agonist LY379268 strongly decreased evoked EPSCs, polysynaptic IPSCs, and frequency, but not amplitude, of spontaneous IPSCs. LY379268 had only weak inhibitory effects on monosynaptic IPSCs and no apparent effect on miniature IPSCs (in TTX). LY379268 decreased synaptically evoked spiking (E-S coupling), a measure of neuronal output function. The results suggest that LY379268 mainly acts on glutamatergic terminals to inhibit excitatory synaptic transmission and feedforward inhibition onto pyramidal cells. Activation of mGluR2/3 is not able to increase the output of pyramidal neurons in mPFC. Therefore, these data support our previously proposed strategy of mGluR5 and CB1 co-activation to restore pyramidal cell output in a pain state.

Disclosures: T. Kiritoshi: None. **V. Neugebauer:** None.

Poster

728. Thalamic and Cortical Processing

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National Natural Science Foundation of China U1201223

Title: Cell-specific adaptations in the nucleus accumbens are critical for neuropathic pain

Authors: *W. REN[1], S. BERGER[1], M. V. CENTENO[1], Y. WU[2], X. NA[2], X. LIU[2], D. J. SURMEIER[1], M. MARTINA[1], A. V. APKARIAN[1];

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Abstract: The nucleus accumbens (NAc) as a key component of forebrain limbic circuitry has been implicated in the expression of chronic pain. The principal neurons in the shell of NAc are divided into two classes: D1 dopamine receptors-expressing direct pathway spiny projection neurons (dSPNs) which directly modulates interface nuclei and facilitate goal-directed behavior; D2 dopamine receptors-expressing indirect SPNs (iSPNs), which indirectly modulates interface nuclei and contribute to the suppression of behaviors and aversive outcomes. Recent human studies found that neurons in the NAc encode pain salience and value for pain relief, and the strength of its functional connectivity with the medial prefrontal cortex is predictive of the transition to chronic pain. These findings suggest that the NAc might be critical for the establishment of chronic neuropathic pain. To determine whether the NAc neurons undergoes reorganization, it was examined using electrophysiological and anatomical approaches in *ex vivo* brain slices from mice 5 days following spared nerve injury (SNI) induced by partial sectioning of the sciatic nerve, a well-established model of the persistent neuropathic pain state. Using transgenic mice in which dSPNs and iSPNs respectively expressed red and green fluorescent proteins, SPNs in the NAc shell were visually identified in NAc slice and then whole cell patch clamp recordings were made. SNI animal had significant and selective elevation in the excitability of iSPNs. As the excitability of iSPNs rose, the dendrites of SNI iSPNs shrank and the number of excitatory glutamatergic synapses fell. In contrast, SNI had no effect on neighboring dSPNs. In parallel, SNI increased dopamine transporter expression and lowered extracellular dopamine concentration in the NAc. Furthermore, treatment with levodopa and naproxen in SNI animals virtually eliminated the effects of nerve injury on iSPN excitability, dendritic architecture and synaptic connectivity and blunted the development of pain, suggesting the iSPN adaptations in SNI were driven by the change in dopamine. Our study demonstrates the cell-specific NAc adaptations in the induction of neuropathic pain, suggesting this pain state has clear parallels to the drug addicted state which preferentially engaged dSPNs in NAc. These observations not only provide new insights into the cellular mechanisms underlying the transition to neuropathic pain, but also point the treatment of levodopa and naproxen combination - two safe and approved drugs with potential for prompt clinical use - as a novel therapeutic strategy for pain.

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Poster

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UTHSC Office of Research to RSW

Title: Neurons in the dysgranular region of rat sensorimotor cortex respond to thermonoxious stimuli and play a key role in second/slow pain processing

Authors: *O. V. FAVOROV[1], J. T. RAMSHUR[2], T. D. CHALLENER[3], R. S. WATERS[4]; [1]Biomed. Engin., Univ. North Carolina, CHAPEL HILL, NC; [2]Biomed. Engin., Univ. of Memphis, Memphis, TN; [3]Biomed. Engin., North Carolina State Univ., Raleigh, NC; [4]Hlth. Sci. Ctr., Univ. of Tennessee, Memphis, TN

Abstract: Introduction: Cytoarchitectonic area 3a of primary somatosensory cortex (S1) appears to play a central role in processing C-nociceptor inputs, perception of the 2nd/slow pain, and in some forms of chronic pain as established in non-human primates (Vierck et al., Pain 154:334-344, 2013). Chapin and Lin (J. Comp. Neurol. 229:199-213, 1984) and most recently Cooke et al. (Cereb. Cortex 22:1959-1978, 2012) proposed that the dysgranular zone in rodent sensorimotor cortex is homologous to primate area 3a. Whether this homology extends to nociception is unknown. Methods: We evaluated the possibility that the transitional zone (TZ), which is a dysgranular region in rat S1 located at the transition between M1 and S1, is the functional equivalent of the nociresponsive region of area 3a in primates by recording extracellular spike discharge activity from layer IV neurons in TZ in rats anesthetized with 1.5-2.5% isoflurane. Thermonoxious stimuli, designed to preferentially activate C-nociceptors, were applied by lowering the contralateral forepaw or hindpaw into 47-53 degrees C heated water bath for 5-10s. In 3 experiments, the dysgranular region was inactivated with lidocaine or cooled. Electrolytic lesions were used to identify recording sites. At the end of experiments, animals were perfused, brains were blocked, hemispheres were flattened and cut to reveal the barrel field and surrounding dysgranular zones. Results: 1. Similarly to nociresponsive neurons in primate area 3a, TZ neurons were minimally affected by non-noxious somatosensory stimuli. 2. These same neurons, however, responded vigorously to thermonoxious stimulation, exhibiting prominent slow temporal summation. As long as noxious stimulation continued, the firing rate progressively increased, reached its peak shortly after the termination of the stimulus, and then decayed slowly. 3. Reversible inactivation of TZ and surrounding territories by lidocaine or cooling lowered pain sensibility of the contralateral limbs as measured by rat performance on a water bath foot-withdrawal test. 4. Recording sites that elicited thermonoxious responses were found to lie in the S1 dysgranular region between the forepaw and hindpaw barrel field representations. Conclusion: Our results suggest that, while in primates A-delta nociceptor-based

sharp discriminative pain is mediated by nociresponsive neurons in area 1, whereas C nociceptor-based slow affective pain is mediated by neurons in area 3a, in rats these tasks are performed by neurons located in the barrels and in TZ, respectively. Most importantly, our findings advance the use of rodents as an experimental model for studying 2nd/slow pain.

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Poster

728. Thalamic and Cortical Processing

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Topic: D.08. Pain

Support: Grant 143-06 from the Instituto Israelita de Ensino e Pesquisa Albert Einstein/ Arthur Goldlust.

Title: Differences in fMRI activation of visual areas during a visuomotor task in individuals with or without migraine

Authors: *A. CONFORTO[1], M. F. P. PERES[2], A. L. GONÇALVES[3], J. P. P. PERES[2], V. GUENDLER[2], E. AMARO JR.[2];
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Abstract: Introduction: Light deprivation (LD) can modulate blood oxygenation level dependent (BOLD) activity in the visual cortex, as well as visual and motor cortex excitability in healthy volunteers (HV). Our goal was to compare changes in BOLD activity, compared to regular light exposure (LE), in HV and in patients with migraine (MP) in the interictal state. Methods: Twenty women participated in the study: 10 patients with migraine and 10 control subjects. Each subject participated in two sessions of functional magnetic resonance imaging (fMRI) in a 3 Tesla scanner. Whole-brain axial structural 3D T1 MPRAGE and FLAIR were performed in all subjects. Two sets of 150 functional images were acquired in each subject before and after 30 minutes of LD or LE. Subjects performed a finger-tapping task with the right hand, paced at 1Hz in a block design. Total run time was 5min, during which 5 epochs were sampled. Subjects were instructed to remain with eyes open, fixating the center of a screen, and keep the right hand at rest, with slight wrist flexion. An image representing the right hand was presented, and the finger

that should touch the thumb was highlighted in green at 1 Hz. Image processing and data analysis were performed using the FMRIB software library package FSL. ANOVA analysis with factors TIME (before or after), SESSION (LD or LE) and GROUP (patients with migraine or controls) was implemented using the Design Matrix feature. Results: ANOVARM revealed significant effects of "GROUP" in a cluster in the bilateral cuneus encompassing the superior border of the calcarine sulcus (V1, BA 17) and extraestriate cortex (V2, BA 18). ANOVA did not show significant effects of "TIME", "SESSION" or interactions between these factors. Conclusions: The main finding of this study was the significant difference between migraineurs and controls, in fMRI activation in primary and extrastriate visual cortex during a motor, finger-tapping task cued by visual stimuli and performed in the interictal state. This finding is consistent with evidence of aberrant processing of visual information in migraine. LD did not modulate fMRI activity in subjects with or without migraine.

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Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 739.01/QQ4

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Ceruloplasmin is expressed by brain pericytes

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Abstract: The copper-binding protein ceruloplasmin denotes a major ferrooxidase. While traditionally being considered a protein of hepatic origin secreted into blood plasma, the protein is also synthesized and secreted in other organs, including astrocytes of the brain, in where extracellular ferrooxidase is necessary to mediate oxidation of ferrous iron released from neurons. Concerning understanding of mechanisms for transport of iron into the brain through the blood-brain barrier (BBB), controversies remain and mainly dispute whether iron is transported through the BBB by means of transcytosis of iron-containing transferrin or by detachment of iron inside brain capillary endothelial cells followed by ferroportin-mediated efflux into the brain interstitium. Using various paradigms for polarized co-culture of rat brain capillary endothelial cells with astrocytes and pericytes, which together form the neurovascular

unit (NVU), we show that pericytes like astrocytes are significantly synthesizing ceruloplasmin both on secreted and GPI-anchored forms. We also show that brain capillary endothelial cells express ferroportin which provides evidence for a role for ferrooxidase activity at the BBB and NVU to enable efflux of ferric iron from brain capillary endothelial cells. Pericytes, unlike astrocytes, form intimate contact with the brain capillary endothelial cells and the data presented here indicates that ceruloplasmin secreted by pericytes could be instrumental for providing ferrooxidase to allow oxidation of ferrous iron of the brain capillary endothelial cells to obtain transport further into the brain as ferric iron. Lack of ceruloplasmin leads to free-radical mediated damage and neuronal degeneration as seen in aceruloplasminemia and major neurodegenerative diseases like Alzheimer's and Parkinson's. The lack of ceruloplasmin secretion by cells of the NVU could impair the integrity of the BBB as an important pathogenetic feature in the early events leading to neurodegeneration.

Disclosures: T. Moos: None. **A. Burkhart:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH/NIGMS P20GM103643

Title: Triptan-Induced modulation of claudin-5 Expression: An *in vitro* study

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Abstract: The blood-brain barrier (BBB) is a highly regulated dynamic interface that separates the peripheral circulating blood supply from the central nervous system. Brain capillary endothelial cells form the structural basis of the BBB, and the low selective permeability has been attributed to a number of features including paracellular expression of tight junction (TJ) proteins. Loss of BBB integrity results in CNS ion imbalance and protein extravasation. 22 million Americans are affected by the neurological disorder, migraine. The mechanisms that underlie this disorder are poorly understood. We propose a role for modification in BBB integrity since MRI studies have shown changes in the BBB integrity during migraine. In

addition, hypoxia is a migraine trigger and migraine is co-morbid with multiple sclerosis, conditions that are associated with modified BBB profiles. Serotonin (5-HT_1) receptor agonists, or triptans, are often used in the treatment of migraine. However, use of triptans over an extended period of time can induce a condition termed medication overuse headache. We hypothesize that triptans induce BBB dysfunction and play a critical role in migraine. To test this hypothesis we have treated a brain endothelial cell line (bEnd.3) with several triptans and studied the effect on claudin-5 expression. Sumatriptan treatment induced a dose- and time-dependent reversible loss of claudin-5 expression. Sumatriptan (10 μM) induced a delayed loss of paracellular claudin-5 expression over 7 days, while 200 μM sumatriptan induced loss over 24 h that was restored to paracellular domains by day 7. Naratriptan and zolmitriptan treatment showed a similar dose and time dependent changes in claudin-5 expression, whereas donitriptan had no effect on claudin-5 expression. Modulation of paracellular claudin-5 expression may be mediated through modulation of Akt phosphorylation state. Western blot analysis showed an increase in Akt (Ser308) phosphorylation, but not Akt (Thr473) phosphorylation following sumatriptan exposure. These results suggest a novel mechanism regulating claudin-5 expression through 5-HT_1 receptor subtypes and the PI3K/Akt signaling pathway. A greater understanding of how alterations in the BBB may increase the probability of medication overuse headache will give insight into the underlying pathology of migraine.

Disclosures: C.L. Willis: None. **H.J. Beaulac:** None. **S.A. Brule:** None. **A.I. McGregor:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 739.03/QQ6

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: The investigation of the drug transporter regulated milnacipran in the hippocampal neuronal *in vitro* system of HT22 cells

Authors: *A. TAGUCHI, K. OSADA, T. HAGA, A. MUTO, Y. OGAWA, T. WATANABE, S. ASARI, M. NAKANO, Y. SASUGA, N. YAMAGUCHI;
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Abstract: Milnacipran was a antidepressant (serotonin noradrenaline reuptake inhibitor: SNRI) prescribed to depression patients in Japan and Europa countries, and fibromyalgia in USA. P-

glycoprotein (P-gp) is a 130-kDa adenosine triphosphate (ATP)-dependent drug transport protein of the blood-brain barrier (BBB). Utilizing ATP hydrolysis as an energy source, P-gp belongs to a large and growing group of transmembrane transporters, which are increasingly recognized as an important part of the blood-brain and blood-cerebrospinal fluid (CSF) barriers. P-gp is expressed by and was first discovered in multiple drug-resistant (MDR) cancer cells, but can also be found in normal tissue. P-gp has been demonstrated to influence the absorption, distribution, and elimination of many commonly used drugs. It has, furthermore, been shown that P-gp influences the distribution of drugs across the BBB. The location of P-gp at the BBB is of importance for the delivery of psychotropic drugs such as antidepressant and antipsychotic medications. HT22 cells are the immortalized mouse hippocampal neuronal cell line, phenotypically resembles neuronal precursor cells. We found that the fluorescent milnacipran localize in the HT22 cells by confocal microscope. Therefor, milacipran was considered that input into HT22 cells from the drug transforter, and output from cells through the P-gp. We investigated whether milnacipran becomed the substrate for P-gp and identified the drug transporter that input milnacipran from blood to brain *in vitro* system of HT22 cells.

Disclosures: **A. Taguchi:** None. **K. Osada:** None. **T. Haga:** None. **A. Muto:** None. **Y. Ogawa:** None. **T. Watanabe:** None. **S. Asari:** None. **M. Nakano:** None. **Y. Sasuga:** None. **N. Yamaguchi:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 739.04/QQ7

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NSC101-2321-B182-012

Title: Transcranial focused ultrasound modulates brain perfusion and neural activity in rats

Authors: *Y.-C. CHEN[1], P.-C. CHU[2], Y.-Y. CHEN[3], H.-Y. LAI[4,1], S.-J. SHAU[3], H.-Y. QIU[5], H.-L. LIU[2], Y.-C. PEI[1,4];
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Abstract: Transcranial focused ultrasound (tFUS) can induce blood-brain barrier (BBB) opening. An improved monitoring method is needed to observe BBB integrity and neurophysiological changes in the brain. Our study employed diffusion-weighted imaging (DWI) and somatosensory evoked potential (SSEP) to examine water perfusion and neural activity after tFUS-induced BBB opening, respectively. Rats were randomly assigned to sham control, low tFUS (0.35 MPa), and high tFUS (0.47 MPa) exposure groups. The tFUS exposure groups were sonicated at the left primary somatosensory cortex forelimb area (S1FL) by a 400-kHz focused ultrasound with intravenous injection of microbubbles. DWI was acquired by a 7T magnetic resonance imager, and apparent diffusion coefficient (ADC) map was computed as an indicator of brain perfusion. Electrical stimulation was administrated at the right forelimb to elicit SSEP in left S1FL. As indicated by ADC and SSEP at S1FL, our results showed that tFUS exposure will transiently modulate brain perfusion and neural activities. Specifically, tFUS intensity negatively correlated to ADC value and SSEP amplitude. Furthermore, it takes more time for ADC and SSEP to return to their baseline levels in the high tFUS exposure group. The results indicate that tFUS-BBB opening induces changes in brain water diffusion and neuromodulation. It is possible that the mechanical force inducted by tFUS causes local edema and subsequently decreases oxygenated blood supply and neural activity.

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Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NS056218

American Heart Association Pre-doctoral Fellowship

OUHSC GSA Research Grant

Donald W. Reynolds Foundation

Title: Restoration of hippocampal vascular density following brain irradiation: A role for endothelial progenitor cells

Authors: *W. E. SONNTAG[1], N. M. ASHPOLE[1], J. P. WARRINGTON[2], M. C. MITSCHELEN[1], H. YAN[1], D. SOSNOWSKA[1], T. GAUTAM[1], J. FARLEY[1], A. CSISZAR[1], Z. UNGVARI[1];
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Abstract: Whole brain radiation therapy (WBRT) induces profound cerebral microvascular rarefaction throughout the hippocampus. Despite the vascular loss and localized cerebral hypoxia, angiogenesis fails to occur, which subsequently induces long-term deficits in learning and memory. The mechanisms underlying the absence of vessel recovery following WBRT are unknown. In this study, we tested the hypotheses that vascular recovery fails to occur as a result of loss of angiogenic drive in the circulation, chronic tissue inflammation, and/or impaired endothelial cell production/recruitment. Ten week old C57BL/6 mice were subjected to a clinical series of fractionated WBRT: 4.5Gy fractions twice a week for 4 weeks. Following WBRT, angiogenic factors in circulation were assessed *in vitro*. Plasma from radiated mice increased *in vitro* endothelial cell proliferation and adhesion to a greater extent than plasma from control mice, indicating WBRT did not suppress the pro-angiogenic drive. Analysis of cytokine levels within the hippocampus revealed that IL-10 and IL-12(p40) were significantly increased one month following WBRT; however, systemic hypoxia (which has been shown to promote vessel recovery) did not reduce these inflammatory markers. Enumeration of endothelial progenitor cells (EPCs) in bone marrow and circulation indicated that WBRT reduced EPC production which was restored with systemic hypoxia. Furthermore, using a bone marrow transplantation model, we determined that bone marrow derived endothelial-like cells home to the hippocampus following systemic hypoxia and contribute to the restoration of vasculature. Thus, the loss of production and homing of EPCs have an important a role in the prolonged vascular rarefaction following WBRT.

Disclosures: W.E. Sonntag: None. **N.M. Ashpole:** None. **J.P. Warrington:** None. **M.C. Mitschelen:** None. **H. Yan:** None. **D. Sosnowska:** None. **T. Gautam:** None. **J. Farley:** None. **A. Csiszar:** None. **Z. Ungvari:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Program#/Poster#: 739.06/QQ9

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Seed Grant Boonshoft SOM 226119

Title: Function of NKCC1 cotransporter in choroid plexus epithelial cells

Authors: *J. M. CRUM[1], F. J. ALVAREZ-LEEFMANS[2];
[1]Neurosci, [2]Pharmacol. & Toxicology, Wright State Univ., Dayton, OH

Abstract: Choroid plexus epithelial cells (CPECs) secrete cerebrospinal fluid (CSF) and regulate its electrolyte composition. CPECs express Na^+/K^+ ATPase and Na^+-K^+-$2Cl^-$cotransporter 1 (NKCC1) on their apical membrane (CSF-facing), deviating from typical basolateral membrane location in secretory epithelia. Given this unique location of NKCC1 in a secretory epithelial cell, the direction of net ion fluxes mediated by this cotransporter, and associated water fluxes, under physiological conditions is controversial in CPECs. Intracellular ion concentrations are a major determinant of the direction of NKCC1-mediated fluxes, with the concentration of intracellular Na^+ ($[Na^+]_i$) and that of extracellular K^+ ($[K^+]_o$) having the greatest impact. Reported $[Na^+]_i$ values range from 10mM in amphibian (Saito & Wright, 1987) to 48mM in rodent (Johanson & Murphy, 1990) choroid plexus. Such a discrepancy in $[Na^+]_i$ has fueled the controversy over the direction of NKCC1-mediated ion fluxes in CPECs, given that the CSF $[K^+]_o$ is 2.9mM and NKCC1 is working near to its flux reversal point. Using the sodium indicator dye Asante Natrium Green-2-AM (ANG-2, TEFLabs), we performed *in vitro* measurements of $[Na^+]_i$ in acutely dissociated single CPECs. Dye calibration was performed for each cell using gramicidin. Results: The $[Na^+]_i$ of CPECs is 9.5 ± 0.5mM, which suggests that NKCC1-mediated fluxes are inwardly directed in CPECs under physiological conditions.

Disclosures: J.M. Crum: None. **F.J. Alvarez-Leefmans:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Program#/Poster#: 739.07/QQ10

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant P01 HD39386

Title: A novel model for brain iron uptake: Introducing the concept of regulation

Authors: *K. A. DUCK[1], P. PONNURU[3], E. B. NEELY[1], M. E. KLINGER[2], I. A. SIMPSON[2], J. R. CONNOR[1];

[1]Neurosurg., [2]Neural and Behavioral Sci., Penn State Hershey Med. Ctr., Hershey, PA; [3]Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Many common neurological disorders such as Alzheimer's, disease Parkinson's disease and Restless Legs Syndrome involve a loss of brain iron homeostasis. Moreover, iron deficiency is the most prevalent nutritional concern worldwide with many associated cognitive and neural ramifications. Therefore, understanding the mechanisms by which iron enters the brain and how those processes are regulated may help to address significant global health issues. The existing paradigm assumes that the endothelial cells forming the blood-brain barrier (BBB) serve as a passive conduit and that transferrin transcytosis is the primary delivery mechanism for brain iron uptake. This theory has many significant flaws. Most notably, it fails to account for the iron requirements of the highly metabolic endothelial cells and does not allow for mechanisms regulating brain iron uptake. We demonstrate that endothelial cells of the BBB, far from serving as a simple conduit, are capable of retaining and releasing iron in response to signaling peptides in an *in vitro* BBB model. Furthermore, *in vivo* studies confirm the presence of radiolabeled iron in the brain microvasculature of mice 24 hours and 5 days after injection of transferrin-bound, radiolabeled iron. These experiments are the first, to our knowledge, to consider the brain and its microvessels as distinct compartments when measuring brain iron uptake. This is significant because iron within the BBB is not readily available to the cells of the brain as our cell culture studies demonstrate. The regulation of brain iron uptake represents a novel direction for study into the distribution of iron in diseases where brain iron imbalance occurs.

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Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Program#/Poster#: 739.08/QQ11

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: FWO Flanders Grant G029811N

Title: Role of connexins and cytoplasmic calcium concentration in controlling blood-brain barrier permeability

Authors: *L. G. LEYBAERT, M. DE BOCK;
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Abstract: The blood-brain barrier (BBB) is formed by capillary endothelial cells of the brain and is a permeability barrier and transport hub for ions, molecules and metabolites. Its restrictive nature is lost in a wide range of brain diseases but the mechanisms of how this occurs is unclear. The calcium ion concentration ([Ca2+]i) in endothelial cells is a major factor influencing BBB function. Connexins form Ca2+-permeable channels that are also influenced by [Ca2+]i and thus contribute to shape [Ca2+]i dynamics. For example, gap junctions that connect the cytoplasm of adjacent cells and hemichannels that are membrane channels not incorporated into gap junctions contribute to intercellular Ca2+ wave propagation. Here, we sought to better understand how connexin channels contribute to [Ca2+]i dynamics and BBB permeability under inflammatory conditions. Bradykinin (BK), an early inflammatory mediator, triggered endothelial Ca2+ oscillations and an increase of permeability in an *in vitro* BBB model. Interestingly, Ca2+ oscillations and permeability increase were blocked by inhibiting connexin channels with Gap27 peptide that targets the vascular connexins Cx37 and Cx43. *In vivo* i.v. injections of BK also increased BBB permeability and this was prevented when Gap27 was co-injected with BK. Mechanistic analysis showed that hemichannels contribute to Ca2+ oscillations by providing an endothelial Ca2+ entry pathway that is controlled by [Ca2+]i. The basic oscillator is formed by ER-located IP3 receptor channels while hemichannels provide a Ca2+ entry pathway that is essential to sustain the oscillations and mediate the oscillation-dependent decrease of BBB permeability. Recent *in vivo* work with i.v. injection of lipopolysaccharide (LPS) as a strong, long-lasting inflammatory stimulus, demonstrated that Gap27 (administered together with LPS) prevented the BBB permeability increase up to 24 h after LPS injection. Interestingly, specific inhibition of Cx43 hemichannels with TAT-Gap19 peptide (administered with LPS) only inhibited the BBB permeability increase at 6 h after LPS injection but not at earlier (3h) or later (24h) time points indicating involvement of Cx43 hemichannels at the 6 h time window. Collectively, these results identify connexins, which co-reside with tight and adherens junctions at the junctional complex, as a novel target to prevent BBB opening under inflammatory conditions.

Disclosures: L.G. Leybaert: None. **M. De Bock:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Program#/Poster#: 739.09/QQ12

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Mayo Clinic Alzheimer's Disease Research Center Grant

Title: Low-density lipoprotein receptor-related protein-1 regulates pericyte-mediated cerebrovascular functions

Authors: *Y. YAMAZAKI, V. FASOLINO, C.-C. LIU, G. BU, T. KANEKIYO;
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Abstract: Pericytes are vascular mural cells of the neurovascular unit, which encase the endothelium of blood capillaries. They regulate multiple cerebrovascular functions including maintenance of the blood-brain barrier (BBB), regulation of vascular stability and control of angiogenesis. Since cerebrovascular system plays a critical role to maintain brain homeostasis, the dysfunction of pericytes is likely involved in several neurological diseases such as vascular cognitive impairment and Alzheimer's disease. The low-density lipoprotein receptor-related protein-1 (LRP1) is a large endocytic receptor abundantly expressed in pericytes. It binds more than 40 ligands including apolipoprotein E, α2-macroglobulin, tissue-type plasminogen activator and matrix metalloproteinases (MMPs). LRP1 also regulates the strength of several signaling pathways by coupling with other cell surface receptors. While emerging evidence demonstrates that LRP1 in neurons and glial cells plays critical roles in maintaining brain homeostasis, it remains unclear how LRP1 in pericytes contributes to cerebrovascular function. Thus, we have explored the roles of LRP1 in pericytes using *in vitro* cellular and *in vivo* animal models. Using knockdown method with siRNA, we found that deletion of LRP1 in human vascular pericytes suppressed cell migration and the formation of capillary-like structure by disturbing PDGF signaling pathway. In addition, *in vitro* BBB model consisting of human primary endothelial cells and pericytes showed that suppression of LRP1 expression in pericytes decreased the transepithelial electrical resistance (TEER) and tight junction (TJ) protein expression by disturbing the metabolism of metalloproteinases (MMPs). Importantly, the expression levels of TJ proteins were significantly reduced in the brains of smLRP1-/- mice which lack LRP1 specifically in vascular mural cells. Cerebrovascular structures were also altered in smLRP1-/- mice. Together, our results demonstrate that LRP1 in pericytes plays important roles in maintaining cerebrovascular functions including angiogenesis and BBB regulation. Thus, LRP1 might serves as a novel therapeutic target for cerebrovascular diseases.

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Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Title: Ferumoxytol phagocytosis and localization in acute neuroinflammation

Authors: *H. MCCONNELL, G. PISHKO, R. WOLTJER, L. MULDOON, E. NEUWELT; Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Hematogenous macrophages and resident microglia will phagocytose ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles when challenged with a neuroinflammatory stimulus. These iron-laden cell populations will traffic to inflammatory lesions in the central nervous system, and this allows the lesions to be monitored *in vivo* using non-invasive magnetic resonance imaging (MRI) techniques. We used immune-competent rats to create a model of localized inflammatory lesion using stereotactic injection of (inflammatory) human tumor cells into the caudate nucleus. At an inflammatory lesion, the USPIO nanoparticle ferumoxytol perturbs the nuclear spin relaxation times of local tissue protons, making it a useful contrast agent for MRI and lesion discrimination. Ferumoxytol is administered systemically and can be localized in tissues using MRI, Perls histochemistry, or an antibody directed against its carboxymethyldextran coating, anti-DX1. Using a peripheral blood smear technique and immunohistochemistry, we found that ferumoxytol nanoparticles were taken up in the periphery by circulating monocytes within 12 hours of systemic administration, and were subsequently found in microglia/macrophages within the neuroinflammatory lesion in the brain. Further, we found that nanoparticles were present extracellulary outside the lesion, and in areas that colocalized with the astrocyte marker GFAP. MRI may be able to differentiate intracellular versus extracellular localization of these nanoparticles. Since monocytes are known to traffic across the blood-brain barrier (BBB) during neuroinflammation, we believe these nanoparticles

may serve as a useful tool for studying BBB permeability changes, diapedesis, and inflammation-induced phagocytosis in the brain. Using these nanoparticles, we are able to perform early dynamic and high resolution MRI to assess relative cerebral blood volume and cerebral blood flow during neuroinflammation. Here we show how the pharmacokinetics, uptake, and fate of these nanoparticles progress over time in the inflamed brain.

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Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

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University of Wisconsin-Madison School of Pharmacy

University of Wisconsin-Madison Graduate School

Title: Examining the roles of diffusion, convection, and perivascular transport in the whole brain distribution of intrathecally applied antibody-based therapeutics

Authors: *M. E. PIZZO[1,2], D. J. WOLAK[1,2], R. G. THORNE[1,2,3,4,5];
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Abstract: Transport processes governing the distribution of macromolecules between the cerebrospinal fluid (CSF) and brain interstitial fluid are not currently well understood (Wolak & Thorne. *Mol Pharm* 2013). Current theories suggest transport is predominantly diffusive (e.g., in the interstitial space; Sykova & Nicholson. *Physiol Rev* 2008; Thorne et al. *PNAS* 2008) or

convective (e.g., in CSF compartments; Iliff et al. *J Clin Invest* 2013; Iliff et al. *Sci Transl Med* 2012). Recent work exploring the fate of macromolecules infused into the CSF of rodents has also suggested a substantial flow into and out of the brain may occur along perivascular spaces (PVS) of cerebral blood vessels (Iliff et al. 2013; Iliff et al. 2012), with possible significance for the clearance of metabolites or toxic proteins (Xie et al. *Science* 2013), brain homeostasis, immune function, and drug delivery. Here, rats received intrathecal infusions of fluorescently labeled antibodies in full length, Fab, or single domain forms, and whole brain distribution was studied using *ex vivo* fluorescence methods. Quantitative diffusion measurements were also performed with the same labeled molecules after intraparenchymal injection using *in vivo* integrative optical imaging (Thorne & Nicholson. *PNAS* 2006). Intrathecal infusions resulted in macromolecule diffusion from the subarachnoid space into the parenchyma across the pial brain surface that varied between areas, e.g., larger signal gradients were more evident ventrally than dorsally. Fluorescence intensity profiles measured along lines normal to the surface of 100 μm brain slices were fit to appropriate models (e.g., Patlak & Fenstermacher. *Am J Physiol* 1975) to determine interstitial diffusion coefficients. Diffusion coefficients obtained from whole brain distributions after intrathecal infusions were then compared to diffusion coefficients from integrative optical imaging after focal input into brain extracellular space. Our results also confirmed that the PVS plays a significant role in whole brain distribution following intrathecal infusion. Molecules were observed in the PVS of deep brain regions within 1 hr, suggesting convective flow in these compartments. For all molecules studied, signal was particularly high around large caliber arteries. Larger molecules appeared more confined to the PVS and did not penetrate far into the parenchyma. Our work extends recent findings examining the relative importance of diffusive and convective transport from the CSF into brain; these insights may have high relevance for the clinical application of CNS targeted biologics, particularly when applied centrally to bypass the blood-brain barrier.

Disclosures: **M.E. Pizzo:** None. **D.J. Wolak:** None. **R.G. Thorne:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 739.12/QQ15

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Newport Equities, LLC

Title: Recurrent Streptococcus pyogenes infections induce Th17 cell infiltration into the CNS via an olfactory route and promote blood-brain barrier damage and IgG deposition

Authors: *M. PLATT[1], T. DILEEPAN[2], D. KNOWLAND[1], E. D. SMITH[1], M. HSU[1], P. CLEARY[2], D. AGALLIU[1];
[1]Developmental and Cell Biol., UC Irvine, Irvine, CA; [2]Microbiology, Univ. of Minnesota, St Paul, MN

Abstract: Repeated intranasal Streptococcus pyogenes [Group A Streptococcus (GAS)] infections induce autoimmune complications in the central nervous system (CNS) in a subset of children that include Sydenham's chorea or Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus (PANDAS). Symptoms of PANDAS include separation anxiety, tics, obsessive-compulsive behavior, and impaired fine motor coordination. Mimic autoantibodies against several neuronal targets have been identified in these disorders; however, the mechanisms by which antibodies cross the blood-brain barrier (BBB) are unknown. Autoimmune consequences of this disease can be modeled in the mouse with recurrent intranasal (i.n.) GAS inoculations. Mice were inoculated i.n. once weekly with either GAS or PBS for five weeks. MHCII tetramers directed at GAS-specific T cells and a biocytin-TMR tracer were used to track T cells and assess BBB integrity, respectively. GAS-specific Th17 cells entered the brain of GAS-inoculated animals and disrupted the BBB to allow deposition of IgG. GAS bacteria were not detected in brains of inoculated mice. The rostrocaudal distribution of T cells suggests migration from the nose-associated lymphoid tissue into the olfactory bulb (OB) along olfactory sensory axons, and then dispersion into other CNS regions. The T cell infiltration into the CNS was associated with enhanced BBB permeability in selected areas, including olfactory bulb, lateral hypothalamus, and amygdala. The increase in BBB permeability in these regions correlated with abnormalities in endothelial tight junction (TJ) integrity such as presence of protrusions or TJ protein degradation leading to formation of gaps. In addition, we found a robust increase in the number of activated microglia in the OB where large numbers of GAS-specific Th17 cells reside after repeated GAS infections.. Finally, we analyzed the behavioral consequences of these neural changes using the Open Field Maze, Elevated Beam Walking challenge, and Induced Grooming tasks. Overall, these findings not only provide a novel breakthrough for understanding how recurrent GAS infections may impair brain function leading to motor and neuropsychiatric disorders, but also suggest a more general mechanism by which other infectious agents that induce Th17 immunity could exacerbate and contribute to neurovascular damage in other CNS autoimmune diseases.

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Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: The National Council of Science and Technology Grant 134592-CONACyT

Title: Inhibition of prolactin with bromocriptine increases blood-brain barrier permeability *in vivo*

Authors: *H. ROSAS-HERNANDEZ[1,2], M. RAMIREZ[1], S. F. ALI[2], C. GONZALEZ[1];
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Abstract: Prolactin (PRL) polypeptide hormone produced by the lactotrophs in the anterior pituitary, is usually related with milk production; however, exert more than 300 actions, including the cellular permeability. The role of PRL as mediator of the *in vivo* brain endothelial permeability and the kind of tight junctions (TJs) involved has been poorly studied, and moreover, its physiological impact upon the maintenance of the blood-brain barrier (BBB), a frontier constituted mainly by endothelial cells which separate the brain tissue from the circulating substances in the vascular system protecting its cellular structures from potentially harmful substances in the blood. The aim of this study was to evaluate the effect of PRL on the permeability of the BBB *in vivo*. To inhibit PRL production, Wistar rats were treated with bromocriptine (BrCr) (1mg/kg i.p.), or saline (control) for 28 days. LPS (1 mg/kg i.p. for 2 hours) was used as a positive control. The BBB permeability was evaluated by injecting a solution of 2% Evans blue dye (2ml/kg) through the jugular vein and the animals were perfused through the heart after 2 hours. After that, the brain was removed and the Evans blue dye was extracted from the brain tissue with formamide and its concentration was quantified. Bromocriptine-treated rats increased the permeability to Evans blue compared to control rats and was no different to the increase of permeability induced by LPS. In association with this finding, we showed that bovine brain microvessel endothelial cells (bBMVECs) treated with PRL, also decreased the permeability and increased trans-endothelial electrical resistance, as a consequence of an increase in the expression of the TJs proteins claudin-5 and occludin. In summary, this data suggests that PRL is a hormone that regulates the permeability of the BBB, possibly by modulating the expression of the TJs proteins. Further investigations are underway to corroborate these findings.

Disclosures: **H. Rosas-Hernandez:** None. **M. Ramirez:** None. **S.F. Ali:** None. **C. Gonzalez:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 739.14/QQ17

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: PROMEP-SEP 14412118

Title: Prenatal stress modifies tight junction protein expression in the rat brain

Authors: ***F. ACOSTA-BLANCO**, J. VELAZQUEZ-MOCTEZUMA, B. GOMEZ; UNIVERSIDAD AUTONOMA METROPOLITANA-IZTAPALAPA, MEXICO, Mexico

Abstract: The blood-brain barrier (BBB) is responsible for maintaining homeostasis in the central nervous system, by modulating the passage of molecules from blood-to-brain. Brain endothelial cells are the major components of the BBB; these cells establish interendotelial tight junctions, which are protein complexes conforming the physical barrier of the BBB. These junctions limit the paracellular diffusion of molecules, forming a continuous and impermeable belt. BBB development begins at the embryonic day 11 (E11) in the rat, and extends until early postnatal life. It has been reported that prenatal stress increases BBB permeability to Evans blue; this increase was related to increased pinocytosis, however, the effect of prenatal stress on endothelial cell tight junctions is unknown. Therefore, we aimed to elucidate the prenatal stress effect on the expression of the tight junction proteins occludin, claudin-5 and zonula occludens-1 in the rat brain. Female Wistar rats and their pups were used. E0 occurred when adult female rats received three ejaculations from a sexual expert male rat. Pregnant rats were randomly assigned to control or prenatal stress group. Prenatal stress animals were immobilized 3 hours daily, from E10-E20.Pregnant dams without stress exposure where used as intact controls. At E18 dams were deeply anesthetized and fetuses were extracted by cesarean, the whole head was obtained. Some other litters were naturally delivered and pups sacrificed at postnatal days 1 and 10, from these animals the brain was removed and olfactory bulbs and hippocampi were dissected and analyzed by immunobloting. The expression of the tight junction protein occludin decreased at E18 in the whole head of prenatally stressed fetuses as compared to intact controls; meanwhile, ZO-1 expression was not affected by prenatal stress at E18. At postnatal days 1 and 10 an increased expression of both ZO-1 and occludin was observed in the olfactory bulb and

hippocampus of prenatally stressed animals. Our results suggest that prenatal stress diminishes tight junction protein expression while it is present, but once removed induces a compensatory effect in tight junction protein expression.

Disclosures: F. Acosta-blanco: None. **J. Velazquez-moctezuma:** None. **B. Gomez:** None.

Poster

739. Blood Brain Barrier: Cell Biology, Physiology, and Disease

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 739.15/QQ18

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: FAPESP

CNPQ

Title: Aerobic training (T) improves blood brain barrier (BBB) integrity in autonomic areas of the spontaneously hypertensive rat (SHR)

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Abstract: It's well known that hypertensive rats exhibit a deficient BBB. To evaluate the combined effect of hypertension and T on BBB integrity within autonomic areas, Adult SHR and WKY rats were submitted to treadmill T (55% of maximum capacity, 1h/day, 5 d/week) or kept sedentary (S) for 8 weeks. At pre-determined times, rats were anaesthetized for chronic catheterization of the femoral and carotid arteries. One day after the surgery, resting arterial pressure (AP) and heart rate (HR) were continuously acquired (40 min) in the conscious freely moving rats. After hemodynamic measurements at rest, rats were anesthetized for carotid infusion with dextrans FITC-10kDA + RHO-70kDA (286µl/100g). Twenty minutes later, rats were sacrificed for brain removal; brains were post-fixed (2 days in 4% paraformaldehyde) and cryoprotected (3 days in 30% sucrose solution). Sequential slices (30 µm) containing the hypothalamic paraventricular nucleus (PVN), the nucleus tractus solitarii (NTS) and the rostral ventrolateral medulla (RVLM) were acquired in a fluorescent microscope and processed by ImageJ analysis. The BBB permeability was evaluated by the capability of the small size fluorescent dextran (FITC10) to partially leak into the brain parenchyma, only if the BBB components were compromised. MAP and HR were significantly increased in SHR-S vs. WKY-

S (MAP: 175±7 mmHg and 128±2 mmHg, respectively; HR: 404±11 bpm and 363±18 bpm, respectively). SHR-T exhibited significant reductions of HR (-9.5% at T2) and MAP (-12.6% at T4), accompanied by increased HR variability (+2.1-fold), decreased pressure variability (-49%) and increased alpha index (+2.3-fold). Dye leakage area was largely reduced at T2 (PVN: from 7.2±1.3 to 1.5±0.3%; NTS: from 13.5±1.3 to 1.5±0.4%; RVLM: 4.7±1.4 to 0.96±0.2). Dye leakage in WKY group (average of 0.63±0.05%) was not changed by T. Data shows a novel beneficial effect of T to maintain BBB integrity and improve perfusion of autonomic brain areas in hypertensive individuals. This adaptive response is crucial for a near normal neuronal activity, thus normalizing autonomic control of the circulation even in the presence of increased pressure. Financial **Support:** FAPESP, CNPq

Disclosures: **L. Buttler:** None. **M.T. Jordão:** None. **A. Ceroni:** None. **L.C. Michelini:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 740.01/QQ19

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Functional connectivity of resting-state networks in young healthy adults

Authors: *S. TANAKA[1], E. KIRINO[2];
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Abstract: Resting-state networks associated with a variety of functions, such as sensorimotor, auditory, visual, cognitive, and emotional information processing were extracted. In contrast to consistent extraction of resting-state networks across the studies, their functional relationships remain unclear. We applied the group independent component analysis on the functional magnetic resonance imaging (fMRI) data taken from 23 healthy adults [12 females and 11 males; age: 18.3 - 23.9 years (mean = 20.9); Japanese; right-handed]. They were kept at rest during the session and were instructed to lay with their eyes closed, think of nothing in particular, and stay awake. The extracted networks were further subjected to cluster and correlational analyses by which we explored their functional relationships. Twenty networks were extracted and were clustered into six groups. The networks within the groups had positive correlations, whereas networks across the groups tended to have negative or no correlations. The default mode network (DMN) had stronger correlations with visual networks than with any other networks. The

salience network was negatively correlated with the DMN, whereas the frontoparietal networks did not show any negative correlations with the DMN. These results suggest that, although participants did not perform any tasks, a variety of information processes progressed during the resting-state fMRI session. Judging from a wide distribution of positive correlations between the DMN and the visual, sensorimotor, and attention networks, resting-state information processing was related to internal visual information processing, such as scene construction, episodic memory recall, and future thinking. Sensory and emotional awareness and self-control were also implicated. These results were consistent with what the participants reported in the post-scan interview.

Disclosures: S. Tanaka: None. **E. Kirino:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 740.02/QQ20

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

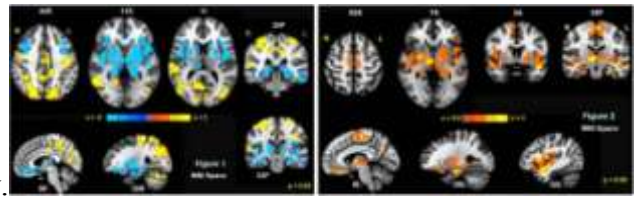
Support: Department of Radiology and Imaging Sciences, Emory University

Title: Anti-hubs and hubs of anticorrelation in high resolution resting state functional connectivity network architecture

Authors: *V. KRISHNAMURTHY[1], R. CABANBAN[2], K. GOPINATH[1];
[1]Radiology and Imaging Sci., [2]Ctr. for Systems Imaging, Emory Univ., Atlanta, GA

Abstract: Previous studies have revealed a number of hubs in whole-brain resting state functional connectivity (rsFC) networks. Here we further characterized global brain connectivity, by probing the rsFC network architecture for the presence of both anti-hubs: nodes with relatively low degree centrality (DC) based on positive cross-correlation coefficient (CC), i.e., exhibiting suppressed resting state activity; and negative hubs: nodes with relatively high degree centrality (DC) based on negative CC, i.e., hubs of anti-correlations that reflect reciprocal modulations in rsFC networks. Normal adults (N = 12; age ~ 27y) were scanned in a 3T MRI. Rest-state fMRI data were acquired with a whole-brain EPI sequence. Each gray matter voxel served as a node in the high-resolution graph analysis. Two separate sets of graphs were constructed employing binarized distance matrices: one based on positive CCs and the other on negative CCs, both thresholded to a sparsity of 0.1. The hubness of each node was determined by

z-transforming the DC maps of each subject. T-tests on these yielded group hubness maps. Significant positive hubs of rsFC based on positive CC (Figure1) were found in a number of areas (e.g. default mode network (DMN) and visual cortex) consistent with previous studies. Anti-hubs (blue-cyan; Figure1) and negative hubs (Figure2) were found in attention network areas (dorsolateral prefrontal cortex (PFC), supplementary motor area), mood network (ventrolateral PFC and subgenual cingulate (ScG)) as well as large parts of hippocampus, basal ganglia and thalamus. The presence of anti- and negative hubs in frontal attention networks and positive hubs in DMN at rest is consistent with reciprocal modulation of these two networks. Results also indicate a dissociation of visceromotor mood network with DMN. Hippocampus, basal ganglia and thalamus are strongly connected to attention and mood networks, which could be the source of their negative or anti-hubness. These hitherto unobserved features in the rsFC network architecture may yield invaluable insight into brain function and connectivity.



Disclosures: **V. Krishnamurthy:** None. **R. Cabanban:** None. **K. Gopinath:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

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Program#/Poster#: 740.03/QQ21

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Bernard Wolfe Health Neuroscience Fund

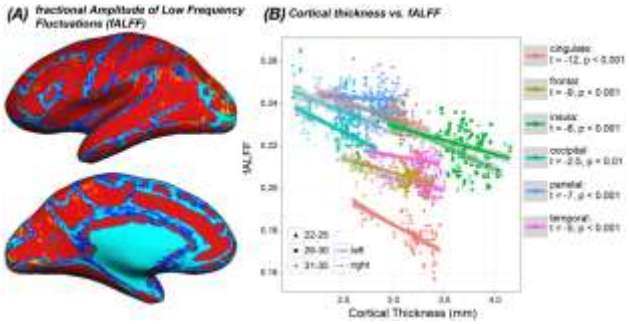
Wellcome Trust

Human Connectome Project, WU-Minn Consortium

Title: Cortical structural correlates of resting-state fMRI power spectra

Authors: ***L. RONAN**, P. C. FLETCHER;
Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Introduction: Low frequency fluctuations (LFF) of the fMRI BOLD signal have been related to spontaneous neuronal activity. By quantifying cortical morphological correlates of resting-state LFF it may be possible to more fully interpret the structural basis of the BOLD signal. In this experiment we demonstrate that cortical thickness is predictive of the regional power spectra of the resting-state fMRI BOLD signal. Methods: Structural and functional data from 63 normal controls (30 males) were taken from the Human Connectome Project. Each subject had a single structural MR image, processed using FreeSurfer (Fischl, 2012) from which cortical thickness measures were derived. Each subject also had two preprocessed repeat resting-state fMRI scans (Glasser, et al., 2013). The power spectra (fractional amplitude of low frequency fluctuations, fALFF (Zou et al., 2008)) of these data were calculated for the frequency range 0.01-0.08Hz using the REST programme (Song et al., 2008). This data was masked for the cortex and mapped to each vertex on the surface. Thereafter the average power spectra per lobe was calculated. Mixed effects models were used to assess the relationship between fALFF and cortical thickness, accounting for the effects of age, hemisphere, sex and repeated measures. Results: Average fALFF was distinct across the different lobes of the brain ($F_{1,1502}=538$, $p < 0.001$). Linear modeling indicated that fALFF was significantly negatively predicted by thickness ($t = -17$, $p < 0.001$). Conclusions: The results of this experiment indicate that cortical morphology is predictive of resting-state fMRI power spectra. Cortical thickness is correlated with neuronal density and cortico-cortical connectivity. As such, these results suggest it may be possible to more fully interpret the BOLD response in terms of cortical cytoarchitecture. This may be particularly relevant for understanding age and disease related-changes of resting-state fMRI, as well as the neuronal basis of resting-state networks which have previously been demonstrated to vary in terms of fALFF (Zou et al., 2008).



Disclosures: **L. Ronan:** None. **P.C. Fletcher:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

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a grant for Exploratory Research (24650226) from the Japan Society for the Promotion of Science

Title: Water diffusion reveals brain networks that modulate multiregional morphological plasticity after repetitive transcranial magnetic

Authors: *M. ABE[1,2], T. MIMA[2];
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Abstract: Repetitive brain stimulation protocols induce plasticity in the stimulated site in brain slice models. Recent evidence from network models has indicated that additional plasticity-related changes occur in nonstimulated remote regions. Despite increasing use of brain stimulation protocols in experimental and clinical settings, the neural substrates underlying the additional effects in remote regions are unknown. Diffusion-weighted MRI (DWI) probes water diffusion and can be used to estimate morphological changes in cortical tissue that occur with the induction of plasticity. Using DWI techniques, we estimated morphological changes induced by application of a 10min-long, subthreshold, low frequency repetitive transcranial magnetic stimulation (rTMS) over the left primary motor cortex (M1). We found that rTMS altered water diffusion in the left M1 and motor-related regions immediately after end of the stimulation. Notably, the change in water diffusion was retained longest (> 10min) in the left M1 and remote regions that had a correlation of baseline fluctuations in water diffusion before rTMS. In contrast, the other remote regions in which baseline fluctuations in water diffusion were not synchronized with the left M1 showed a faster decay in the change in water diffusion (< 10 min). We conclude that synchronization of water diffusion at rest between stimulated and remote regions ensures retention of rTMS-induced changes in water diffusion in remote regions. Synchronized fluctuations in the morphology of cortical microstructures between stimulated and remote regions might identify networks that allow retention of plasticity-related morphological changes in multiple regions after brain stimulation protocols. These results increase our understanding of the effects of brain stimulation-induced plasticity on multiregional brain networks. DWI techniques could provide a tool to evaluate treatment effects of brain stimulation protocols in patients with brain disorders.

Disclosures: **M. Abe:** None. **T. Mima:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: This work was supported by National Research Foundation of Korea(NRF) grant funded by the Korea goverment (MSIP) (No 2011-0030815)

This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MEST) (No. NRF-2013R1A2A1A05006227)

Title: Regional brain metabolism correlated with large-scale functional network: Simultaneously acquired resting state FDG PET/fMRI study

Authors: H. CHOI[1], J. HAHM[1], Y. HUH[1], *Y. KIM[2], H. KANG[1], H. LEE[1], E. E. KIM[1], J.-K. CHUNG[1], D. LEE[1];
[1]Dept. of Nuclear Med., Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [2]Dept. of Nuclear Med., Seoul Natl. Univ. Boramae Med. Ctr., Seoul, Korea, Republic of

Abstract: Regional brain metabolism measured by [^{18}F]fluorodeoxyglucose (FDG) PET is closely related to neuronal activity. In resting state, inter-subject variations in brain metabolism are affected by several cognitive and behavioral conditions. Here, we investigate whether resting state regional metabolic activity affects large-scale functional connectivity using simultaneously acquired FDG PET/fMRI. Twenty-six subjects were enrolled for resting state PET/fMRI on dedicated PET/MR scanner. The functional connectivity of the brain was assessed by fMRI using correlations of pairwise predefined volume-of-interests (VOIs). Using FDG PET, regional metabolism was calculated by the same VOIs. We assessed correlations between metabolic activity and functional connectivity of pairwise VOIs measured by fMRI. Functional brain networks were constructed and we investigated how network topology was changed according to the regional metabolic activity from each of VOIs. The results showed regional metabolic activity was associated with large-scale functional connectivity. Patterns of regional metabolism associated functional connectivity were significantly different between the brain regions. Furthermore, metabolic activity of each VOI is related to different topological properties of individual functional networks. Our results demonstrate the feasibility of simultaneous PET/MRI for functional brain study. Because of spatially and temporally coregistered imaging techniques,

the new approach reveals comprehensive information to integrate regional metabolic activity and global functional networks, which might enlighten about how brain networks work.

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Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 740.06/QQ24

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Obsessive-compulsive disorder results in increased dACC modulation of frontal-striatal-thalamic circuitry during working memory: Network signatures of inefficiency

Authors: *E. HONG[1], A. BURGESS[1], G. HANNA[2], P. ARNOLD[3], V. DIWADKAR[1], D. ROSENBERG[1];
[1]Wayne State Univ., Detroit, MI; [2]Univ. of Michigan, Ann Arbor, MI; [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: Background: Obsessive-compulsive disorder (OCD) is characterized by the dysregulation of frontal-striatal-thalamic circuitry (FSTC) (Roth et al., 2007), yet the network bases of this have not been well characterized. Assuming a central role for dorsal anterior cingulate based mechanisms of control of brain networks (Paus et al., 2001), we investigated dysfunctional dACC modulation of FSTC in OCD during a simple working memory paradigm. Network interactions were investigated examining the effective connectivity (Friston, 2011) of the dACC on FSTC. **Methods:** Twenty-two healthy controls (age:12-21, mean =17.1; 13 males) and 14 OCD (age:13-22, mean =19.2; 3 males) underwent fMRI (3T Siemens Verio) during which subjects performed a verbal working memory paradigm (0-back and 1-back conditions in 30 s epochs, interspersed with 20 s rest epochs). fMRI data were analyzed in SPM8 using typical methods. To assess dACC modulation of FSTC (Friston et al., 1997), its time series was convolved with the contrast of interest (1back > 0back); the resultant first level maps were submitted to second level random effects analysis to identify between-group differences. **Results:** Significantly increased modulation of FSTC was observed in OCD. Peaks within the FSTC were observed in the striatum (t=4.08, x=28, y=9, z=9), and the dorsal prefrontal cortex (t=3.2, x=-34,y=36, z=16). Additional peaks were observed in the parietal cortex (t=3.81, x=18, y=-57, z=60). **Conclusion:** The dACC is a prime target for structural MRI studies in OCD

(Radua & Mataix-Cols, 2009) and recent evidence points to increased engagement in response to increased task demand (Koch et al., 2012). Our results extend previous studies by suggesting that dysfunctional control mechanisms in the dACC exert dysfunctional and inefficient network-based effects in the developing OCD brain. These studies, consistent with system's based approaches to characterizing brain function (Stephan, 2004), motivate the need for understanding network interactions in OCD to arrive at a more comprehensive characterization of the neural correlates of the disorder.

Disclosures: **E. Hong:** None. **A. Burgess:** None. **G. Hanna:** None. **P. Arnold:** None. **V. Diwadkar:** None. **D. Rosenberg:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grants R01 HL102119

NIH Grants R21 DA032022

NIH Grants P30 NS045839

Title: Total sleep deprivation alters resting state amygdala connectivity

Authors: ***Z. FANG**[1,2], N. MA[2], S. ZHU[2], S. HU[2], J. A. DETRE[2], D. F. DINGES[3], H. RAO[2,3]; [1]Sun Yat-sen Univ., Guangzhou, China; [2]Ctr. for Functional Neuroimaging, Dept. of Neurol., [3]Div. of Sleep and Chronobiology,Unit for Exptl. Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Sleep deprivation (SD) is associated with exaggerated neural and behavioral reactivity to negative stimuli. Previous studies have demonstrated that the SD significantly amplifies amygdala reactivity in response to negative emotional stimuli and reduces functional connectivity (FC) between amygdala and prefrontal cortex during emotion tasks. In the current study, we used resting state fMRI and examined the effects of one night of acute total SD as well as two nights recovery sleep on resting amygdala connectivity. Methods: Thirty-six healthy adults (18 female, age 21-50 yrs) participated in a 5-day and 4-night SD study. They were scanned three times between 7-9 am on a Siemens 3T Trio scanner at resting state using a

standard EPI sequence. All subjects underwent the three scans in a fixed order: a 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). The bilateral amygdala was selected as the seed region for FC analyses. Data were analyzed by SPM8 and REST toolbox. Results: The FC analyses revealed that SD significantly enhanced amygdala connectivity to the anterior cingulate cortex (ACC) and the dorsal lateral prefrontal cortex (DLPFC) but SD reduced amygdala connectivity to the sensorimotor cortex compared to the BS and RS scans. There was no difference in amygdala connectivity between the BS and RS. Conclusion: The increased resting state amygdala connectivity to the ACC and DLPFC are opposite to previous findings of reduced amygdala connectivity during tasks after sleep loss, suggesting that SD may have differential effects on brain function at rest and during tasks. However, enhanced connectivity between the ACC and amygdala resembles the effects of antidepressant treatments like sertraline on resting state brain connectivity, thus may provide a potential neural bases for the mood improvements after sleep loss in depression patients. Grant **Support:** Supported in part by NIH Grants R01 HL102119, R21 DA032022, and P30 NS045839.

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Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

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Program#/Poster#: 740.08/QQ26

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01-AT004921

NIH Grant R01-HL102119

NIH Grant R21-DA032022

NIH Grant P30-NS045839

Title: Resting CBF changes in the anterior cingulate cortex predict blood pressure reduction after lifestyle modification in mild to moderate hypertension

Authors: *H. RAO[1], R. BHAVSAR[1], A. BOWLER[2], Z. FANG[1], N. MA[1], J. A. DETRE[1], D. L. COHEN[2];

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Abstract: Introduction: Hypertension is a major public health issue affecting millions of people worldwide. Elevated blood pressure (BP) is a major risk factor and predictor of stroke, cardiovascular and chronic kidney diseases. Even modest elevations in BP within the high normal range (Systolic BP 120-139 or Diastolic BP 80-89 mmHg) which fall into the category of pre-hypertension are associated with an increased risk for developing overt hypertension and cardiovascular disease. The anterior cingulate cortex (ACC) is important for regulating cardiovascular reactions to behavioral stressors. Previous studies have showed that greater ACC activation correlated with increased mean arterial pressure (MAP) during stressful tasks. Here we used arterial spin labeling (ASL) perfusion fMRI to non-invasively quantify resting cerebral blood flow (CBF) and examine the associations between resting ACC CBF and MAP in patients with mild to moderate hypertension. Methods: The BP and CBF data were collected from the Lifestyle Modification and Blood Pressure Study (LIMBS II), a phase 2 randomized controlled trial designed to determine the effects of 24 weeks of yoga therapy and/or enhanced lifestyle modification on lowering blood pressure in in patients with pre-hypertension and stage 1 hypertension. A total of 57 subjects (27 females, ages 23-71 years) were scanned at rest using a pseudo-continuous ASL sequence on a Siemens 3T MR scanner. BP measures were obtained by 24 hour ambulatory blood pressure monitoring outside the MR scanner. Forty-one of 57 subjects also had BP measures and ASL scans after the intervention. Results: Subjects showed significantly reduced MAP after yoga therapy and/or enhanced lifestyle modification (101.5 ± 6.4 vs. 98.6 ± 6.6 mmHg, $p < 0.001$). At baseline before the intervention, regional CBF in the perigenual ACC correlated with MAP ($r = 0.41$, $p = 0.001$). Moreover, regional CBF changes in the perigenual ACC predicted MAP reduction after the intervention ($r = 0.43$, $p = 0.005$). Conclusion: The present study demonstrates that resting CBF in the ACC correlates with MAP at baseline, and that ACC CBF changes predict MAP reduction after 24 weeks of yoga therapy and/or enhanced lifestyle modification in patients with mild to moderate hypertension. These findings extend previous PET and BOLD fMRI studies showing positive associations between MAP and ACC activity during different behavioral stressors to the resting state. The results provide further evidence supporting that the cingulate cortex plays a key role in calibrating blood pressure and other cardiovascular reactions to meet the metabolic demands of cognitive and emotional challenges.

Disclosures: H. Rao: None. **R. Bhavsar:** None. **D.L. Cohen:** None. **Z. Fang:** None. **N. Ma:** None. **J.A. Detre:** None. **A. Bowler:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

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Program#/Poster#: 740.09/QQ27

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NICHD 5U54HD061221

Title: Reduced neural activity in patients with ornithine transcarbamylase deficiency during a Stroop task

Authors: *I. M. PACHECO-COLÓN[1], A. L. GROPMAN[2,3,4], C. SPROUSE[2,3], G. HELMAN[2], J. W. VANMETER[1];
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Abstract: Ornithine transcarbamylase deficiency (OTCD) is an X-linked urea cycle disorder that causes hyperammonemic episodes leading to white matter injury and impairments in executive functioning (Gropman & Batshaw, 2004). This study seeks to examine differences in neural activity during the Stroop task between OTCD patients and controls. Nine OTCD patients and 13 healthy adults completed a Stroop task during a 3T fMRI scan. Participants responded to the color of the ink, not the color the word reads, by pressing buttons corresponding to each color. Standard processing of the fMRI data was done using SPM8. Linear contrasts examining incongruent greater than congruent were entered into a two sample t-test using age as a covariate. The Stroop interference effect, represented by the difference in reaction time between incongruent and congruent trials, was more pronounced in the performance of OTCD patients than in that of controls. This between-group difference approached significance at $t(20)=1.99$, $p=0.061$, and would likely reach it with a larger sample. There were no group differences in accuracy. Group comparisons of the fMRI Stroop data indicated that OTCD patients showed reduced activation compared to controls for Incongruent-Congruent in the right inferior frontal cortex, right middle temporal gyrus, posterior cingulate cortex (PCC), left putamen, right superior temporal gyrus (rSTG), and right supplementary motor area (SMA) ($p<.005$, unc., $k>20$). Overall, these results indicate that despite equivalent performance on the task the OTCD patients had slower reaction times and were less efficient in several task related areas. Inferior frontal regions have been implicated in the Stroop task, as they are involved in the inhibition of reading (Leung, Skudlarski, Gatenby, Peterson, & Gore, 2000). Reduced activity in this area suggests that OTCD patients had a harder time suppressing the instinct to read, which is in line with their executive function deficits (Gyato et al., 2004). Executive deficits could also be related to the underactivity observed in PCC, implicated in regulating the focus of attention (Leech & Sharp, 2014), and rSTG, involved in integration of previous actions and outcomes into a

decision-making strategy (Paulus, Feinstein, Leland, & Simmons, 2005). Finally, the putamen and SMA are associated with movement regulation (Seitz, Roland, Bohm, Greitz, & Stone-Elander, 1990; Chen, Scangos, & Stuphorn, 2010). Reduced activity of these regions could reflect OTCD patients' deficits in fine motor dexterity and working memory deficits, since they had to remember which button corresponded to which color (Gyato et al., 2004).

Disclosures: I.M. Pacheco-Colón: None. **A.L. Gropman:** None. **C. Sprouse:** None. **G. Helman:** None. **J.W. VanMeter:** None.

Poster

740. Functional Neuroimaging: Networks, Regions, and Resting State

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 740.10/QQ28

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Human Frontier Science Program (RGP0048/2012)

Centre for Integrative Neuroscience and Neurodynamics, University of Reading

Title: Choline levels in human parietal cortex predict performance in a visuospatial attention task

Authors: M. LINDNER[1], S. IQBAL[1], P. G. MULLINS[2], *A. CHRISTAKOU[1];
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Abstract: Introduction Acetylcholine (ACH) involvement in visuospatial attention is well-documented in both animal and human research. Levels of ACH can be pharmacologically manipulated in both animals and humans, but it is not possible to measure brain ACH directly in humans *in vivo*. A key part of the ACH biochemical cascade in neural tissue is choline (CHO), which is "visible" in the chemical spectra acquired with magnetic resonance spectroscopy (MRS). There is tentative evidence in the literature that levels of CHO may be an indirect but proportional measure of ACH availability in brain tissue. In this study we measured CHO levels in the parietal cortex using MRS during performance of a visuospatial attention task, where the involvement of ACH in the parietal cortex is well characterised. Methods We used MRS as a functional measure, acquiring non-averaged single voxels in the parietal cortex (TR=2.5s), while subjects performed a simple visuospatial attention task. Subjects had to covertly shift attention to

one of two gratings following a cue, and report its rotation relative to the vertical. In a control condition subjects had to simply press a button when the gratings appeared. Trials lasted 5s, allowing two spectrum acquisitions (volumes) per trial. We averaged the water-referenced spectra for ipsilateral (shift in the acquisition hemispace), contralateral (shift in the opposite hemispace), and control conditions for the two volumes separately. After preprocessing and quantification of the averaged data, we calculated the difference in CHO between the late and early volume of each condition. Results When the shift direction was contralateral to the MRS voxel, early volume CHO was increased and late volume CHO was decreased relative to the ipsilateral and control conditions (interaction $p<0.5$). This drop in CHO between early and late volumes in the contralateral (but not ipsilateral or control) condition was associated with increased task accuracy ($r=-.62$, $p<.05$). Conclusions We demonstrate that parietal CHO levels are associated with shifting visual attention in contralateral space. We reasoned that as more ACH is required in the contralateral condition, more ACH will be produced to refill the vesicles. Free CHO will therefore be extracted from the membrane, so CHO is higher in the early volume when the attentional shift is taking place, and lower in the late volume when it has been used to replenish ACH. This is in line with our results showing that a drop in CHO following a visuospatial attention shift in contralateral space predicts task performance. Our results strengthen the proposal that CHO MRS can be used as a functionally-relevant proxy measure of brain ACH *in vivo*.

Disclosures: **M. Lindner:** None. **S. Iqbal:** None. **A. Christakou:** None. **P.G. Mullins:** None.

Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R01AG039103

NIMH Grant R01MH07458

Title: Age-related differences in event-related functional connectivity during associative encoding

Authors: ***E. D. HORNE**[1], M. DE CHASTELAINE[2], D. R. KING[2], M. D. RUGG[2];
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Abstract: Positive subsequent memory effects (SMEs) refer to higher levels of encoding-related activity for later remembered than later forgotten study items, and negative SMEs refer to lower levels of encoding-related activity for later remembered items. Whereas numerous studies have described age-related differences in SMEs, few have investigated the effects of age on encoding-related modulation of functional connectivity. Here, fMRI data were obtained from 36 young, 36 middle-aged, and 64 older adults while they studied a series of word pairs. On a later associative recognition test, the participants discriminated between 'intact' (same pairing as at study), 'rearranged' (different pairing from study) and new pairs. Associative recognition performance declined monotonically across the three age groups. SMEs were identified by contrasting the activity elicited by the study pairs according to whether the pairs were correctly judged intact or were incorrectly endorsed as rearranged on the subsequent memory test. Ten seed regions were selected, centered on five positive and five negative SMEs identified by this contrast. Psychophysiological interaction (PPI) analysis was employed to identify where functional connectivity with each of these regions was modulated as a function of later memory performance, as well as to identify possible age-related differences in encoding-related modulation of connectivity. Across the three age groups, numerous cortical and sub-cortical regions demonstrated greater connectivity with the 10 seed regions during presentation of study items that went on to receive accurate rather than inaccurate associative recognition judgments. Importantly, these common effects were accompanied by age-related differences in encoding-related connectivity changes. In young adults, increased connectivity in association with successful encoding was evident between all 10 seed regions and medial prefrontal cortex (mPFC). These effects were significantly weaker in older subjects, in whom increases in connectivity with the mPFC were weak and inconsistent. Similar between-group differences were observed when encoding-related connectivity increases were contrasted between middle-aged and older participants, albeit for a smaller number of seed regions (seven regions instead of nine). Thus, relative to their young and middle-aged counterparts, successful associative encoding in older individuals is accompanied by reduced functional connectivity between the mPFC and a widely distributed set of cortical regions manifesting both negative and positive SMEs.

Disclosures: E.D. Horne: None. **M. de Chastelaine:** None. **D.R. King:** None. **M.D. Rugg:** None.

Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 741.02/QQ30

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R01AG039103

Title: The relationships between age, negative subsequent memory effects and task-negative effects during associative memory encoding

Authors: *M. A. DE CHASTELAINE[1], J. T. MATTSON[2], T. H. WANG[1], B. DONLEY[1], M. D. RUGG[1];
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Abstract: Negative subsequent memory effects - lower study activity for later remembered than later forgotten items - are often attenuated in older adults. Brain regions identified by negative subsequent memory contrasts largely fall within the 'default mode network' - a set of regions that exhibit greater activity during 'rest' than during task engagement. Such 'task-negative' activity is also reduced in older compared to younger individuals. The present fMRI experiment used an associative recognition task to investigate the relationships between age, negative subsequent memory effects and task-negative effects. Young, middle-aged and older adults (total n = 136) were scanned while they made relational semantic judgments on visually presented word pairs. Participants later made associative recognition judgments on studied, rearranged (items studied on different trials) and new pairs. fMRI negative subsequent memory effects were operationalized as greater activity for studied pairs incorrectly endorsed as rearranged than those correctly endorsed as intact. Task-negative effects were identified by contrasting activity elicited by studied pairs (regardless of how endorsed) with respect to the implicit baseline of the GLM. Most regions demonstrating negative subsequent memory also demonstrated task-negative effects although negative subsequent memory effects were also evident in one task-positive region (insula). There was no relationship between the size of negative subsequent memory and task-negative effects across participants. While negative subsequent memory effects showed a monotonic attenuation with age (young > middle-aged > older), task-negative effects were markedly reduced from the young to the middle-aged group, but showed no further reduction in the older group. Across groups, regression analyses identified age, but not memory performance, as a strong predictor of the size of both negative subsequent memory effects and task-negative effects. Further analyses showed that, in the older group only, task-negative effects predicted later memory performance independently of both age and negative subsequent memory effects. The magnitude of negative subsequent memory effects did not predict memory performance in this or the other age groups. Together, these findings indicate a functional dissociation between negative subsequent memory effects and task-negative effects in default mode regions with respect to encoding efficacy. Task-negative effects at encoding, but not negative subsequent memory effects, predicted higher associative memory performance in older individuals.

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Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 741.03/QQ31

Topic: F.01. Human Cognition and Behavior

Title: The neural representation of space-time in remembered experience

Authors: ***V. SREEKUMAR**[1], T. SMITH[1], D. NIELSON[1], S. DENNIS[2], P. SEDERBERG[1]; [1]Ohio State Univ., Columbus, OH; [2]Univ. of Newcastle, NSW, Australia

Abstract: Episodic memory enables us to remember experiences by engaging in mental space-time travel (Tulving, 1993). Here, we identify the brain regions that track spatial, temporal and spatio-temporal distance relationships between personal experiences and thereby support mental space-time travel during autobiographical memory (AM) reconstruction. Nine participants used android phones equipped with custom lifelogging software to collect data about their everyday events for a period of 4 weeks. The phone automatically captured images, GPS, time and other sensor based data. Participants were brought into the lab 7-14 days after the data collection phase for a reminiscence task in an fMRI scanner. Each participant viewed his/her own images and mentally relived the corresponding events. We employed representational similarity analysis (RSA) to identify the regions that support AM reconstruction by preserving spatial, temporal and spatio-temporal distance relationships between event representations for the images the participants rated as inducing vivid memories. The network that preserved spatial (GPS) distances between events during AM reconstruction included regions that are involved in spatial imagery during AM (e.g. posterior cingulate and parahippocampal gyrus), vividness of AM (e.g. right occipital fusiform gyrus) and semantic aspects of AM (e.g. lateral temporal gyrus). The network that tracked temporal distances between events included regions that are involved in successful memory retrieval (e.g. right frontal pole), vividness of visual imagery (e.g. precuneus), and context (e.g., parahippocampal cortex). Finally, the regions that correlated with the interaction between spatial and temporal distances included the left subcallosal cortex (which is connected to the hippocampus, DLPFC and OFC and is implicated in Alzheimer's disease) and the right anterior hippocampus. These results suggest that these networks that represent the

spatio-temporal content of remembered experiences combine to provide us with a way to engage in mental space-time travel during successful AM reconstruction.

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Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NRF-2010-0018949

Title: The activity in the default-mode network region can predict subsequent forgetting

Authors: *K. TARK[1], H. LEE[2], Y. BAK[1], D.-J. YI[1];
[1]Psychology, Yonsei Univ., Seoul, Korea, Republic of; [2]Psychology, New York Univ., New York, NY

Abstract: It has been investigated how the human brain constructs long-term memories by comparing neural responses during encoding successfully remembered and forgotten stimuli in the later recollection. In the present study, we aimed to see which brain regions are associated with incidental subsequent remembering or forgetting using functional magnetic resonance imaging (fMRI). While scanning, participants (N=17) responded to the direction of a presented arrow, preceded by a face or a scene image. After scanning, they performed a surprising subsequent memory test for the incidentally learned faces and scenes. We found that BOLD responses during encoding remembered scenes were greater than forgotten scenes in parahippocampal place area (PPA), but such a difference was not observed in face fusiform area (FFA). On the other hand, greater neural responses were evoked while encoding subsequently forgotten items rather than remembered items in the default-mode network regions (DMN; i.e., medial prefrontal cortex, posterior cingulate cortex). Next, to see if the multi-voxel patterns of activities differ while encoding subsequently remembered vs. forgotten items, we computed the correlation between neural patterns in ventral visual cortex and DMN as an index of neural similarity. The result showed that the correlation was higher for remembered faces and scenes, compared to the forgotten in FFA and PPA, respectively. However, in DMN, we found no difference of pattern similarities between during encoding remembered and forgotten items. Furthermore, the multi-voxel patterns of activity in DMN failed to predict whether the item would be successfully recollected or not. Our results suggest that indistinguishable patterns of

neural responses in DMN are involved in encoding to-be-remembered and to-be-forgotten items, but the amplitude of those patterns may determine whether the item is later remembered or forgotten.

Disclosures: **K. Tark:** None. **H. Lee:** None. **Y. Bak:** None. **D. Yi:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NIH training grant #T32MH065214

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Title: Posterior distributions over hidden variables: Schemas in the brain

Authors: ***S. C. CHAN**, Y. NIV, K. A. NORMAN;
Princeton Univ., Princeton, NJ

Abstract: In recent years, there has been renewed interest among cognitive neuroscientists in how memory encoding and retrieval are shaped by situation-specific prior knowledge ("schemas"; e.g., Tse et al., 2007). As work in this area progresses, it is important to clarify exactly what constitutes a schema and how they are formed. Here, we provide a simple definition of schemas in terms of Bayesian latent cause models (e.g., Gershman, Blei, & Niv, 2010), and we use these models to help us localize brain regions that are involved in representing situational knowledge. According to Bayesian latent cause models, situations can be viewed as hidden (latent) causes that give rise to observable events (e.g., the situation of "eating dinner in a restaurant" generates observable events like "being given a menu"), and the term "schema" can be used to refer to knowledge about how a particular hidden cause ("dinner") gives rise to observable events. Given a particular set of observations and a "generative model" specifying how different situations (causes) give rise to observable data, one can use Bayesian inference to infer which situations might have generated the observed data, yielding a posterior probability distribution over the space of possible situations. We hypothesize that some of the brain areas implicated in representing schemas in fact represent a posterior distribution over situations, and that this posterior distribution is updated in the manner specified by Bayesian latent cause models. In our fMRI experiment, we looked for areas of the brain that might encode this

posterior distribution. Subjects were trained to learn the statistics of a "safari" environment, in which animals were unevenly distributed across 4 different "zones". While subjects were scanned, they observed sequences of animals, and were asked to guess which zone the animals had come from. In this way, subjects were required to continuously update a posterior distribution over the hidden variable "zone". We used a Bayesian model to predict how the posterior distribution over zones should change from trial to trial. We then used multivariate pattern similarity analysis to find areas of the brain where the spatial pattern of activity changed from trial to trial in a similar way (i.e., neural patterns were similar when the posterior was similar; neural patterns were different when the posterior was different). Preliminary results suggest that neural similarity structure in medial PFC (and other regions previously implicated *in situ*ation representation: Ranganath & Ritchey, 2012) was better predicted by the posterior distribution over "zones" than by other, competing models.

Disclosures: S.C. Chan: None. **Y. Niv:** None. **K.A. Norman:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NSF GRFP

Title: High discrimination demands reduce interference during later learning

Authors: *S. E. FAVILA, A. J. H. CHANALES, B. A. KUHL;
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Abstract: Many of our experiences share overlapping features. Computational models of episodic memory argue that interference during memory retrieval can be avoided if events are encoded in a manner that minimizes feature overlap. We assessed the role of task demands in this process. We hypothesized that demands to discriminate between perceptually similar stimuli would influence the representational distance between those stimuli and that such changes would predict interference in new learning contexts. To test this idea, we first asked human participants to learn scene-face associations. Critically, the scene images consisted of pairs of perceptually similar scenes, or pair mates. By associating some scene pair mates with different faces and other pair mates with a common face, we structured task demands to require associative discrimination

between some pair mates but not others. Subjects were also exposed to a set of scene pairs that were not included in the associative learning task. These scenes served as a baseline condition for our task demand manipulation. We subsequently scanned subjects while they viewed all of the scenes, allowing us to estimate the neural pattern similarity of pair mates as a function of prior learning demands. Finally, subjects participated in a new learning session that paired each scene with a unique object. Behavioral data from the second learning session showed that subjects were better at learning scene-object associations if scene pair mates had previously been associated with distinct faces, relative to pair mates associated with the same face and baseline pair mates that had no prior associations. Thus, the benefits of high discrimination demands outweighed proactive interference. In particular, these benefits were reflected in a lower probability that subjects would pair a scene with the object that was associated with the scene's pair mate during learning session 2. This suggests that learning demands during the first session specifically influenced whether interference occurred between pair mates during the second session. fMRI analyses showed that perceptual similarity between pair mates was reflected in the correlation of voxel activity patterns in visual cortex; pattern similarity was greater between scene pair mates than unrelated scenes in early and higher-level visual cortical areas. Moreover, learning demands on day 1 differentially influenced pattern similarity across cortical regions. We consider how these modulations of pattern similarity between pair mates relate to behavioral measures of interference during learning on day 2.

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Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: R01 AG030311

Title: Interactions between default and salience networks at rest predict the affective enhancement of memory

Authors: *J. M. ANDREANO[1,2], A. TOUROUTOGLOU[1,2], M. ADEBAYO[1], M. STEPANOVIC[1], C. CASO[1], B. C. DICKERSON[1,2], L. FELDMAN BARRETT[3,1,2]; [1]Massachusetts Gen. Hosp., Boston, MA; [2]Athinoula A. Martinos Ctr. for Biomed. Imaging, Charlestown, MA; [3]Northeastern Univ., Boston, MA

Abstract: Episodic memory and negative affect are thought to be processed by distinct brain networks (the default mode and salience networks, respectively), and previous studies have demonstrated that individual differences in memory or affect relate to individual differences in resting-state functional connectivity (RSFC) within these respective networks. However, behavioral studies clearly indicate that these networks interact, as affectively negative material is consistently better remembered than neutral material. Furthermore, some studies have indicated that neutral material encoded in a negative state is similarly enhanced, although findings on this question are conflicting. No prior studies on affective influences on memory have examined RSFC network-level neural substrates. In this study, we tested the hypothesis that the strength of connectivity between default mode and salience networks (inter-network connectivity) predicts the magnitude of memory enhancement for neutral material caused by the induction of negative affect at the time of encoding. Group analysis showed that negative affect induction during encoding boosted recognition discriminability memory performance, but there were substantial individual differences in this "affective enhancement of memory" effect. Individual differences in RSFC between key nodes of the salience and default mode networks predicted individual differences in the enhancement of neutral memory by negative affect induction, with stronger connectivity predicting greater enhancement. Thus, affective experience during encoding of neutral material enhanced memory more potently in individuals with a greater degree of baseline connectivity between the putatively distinct default mode and salience networks.

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Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 741.08/QQ36

Topic: F.01. Human Cognition and Behavior

Title: Electrophysiological evidence of age-related inter-hemispheric changes during episodic encoding processes

Authors: *D. TROMP, A. DUFOUR, T. PEBAYLE, O. DESPRES;
LNCA, CNRS, Strasbourg, France

Abstract: The aging of the population has shed light on aging-associated cognitive deficits, including deficits in episodic memory (EM), a cognitive system that makes possible the

recording, storage, and retrieval of information about personal experiences and their temporal and spatial contexts. For information to be useful in the future, it must be encoded in memory in a manner allowing its subsequent retrieval. A deficit in encoding may explain why some experiences are remembered whereas others are forgotten. The aim of this study was to analyze early process activity that allows information to be anchored in memory and to compare this activity in two age groups. Behavioral evaluations, combined with electrophysiological studies of human memory, especially event-related potentials (ERP), may provide better insights into the cognitive processes associated with episodic encoding in aging. ERPs of young and older subjects were recorded during a study/test procedure. Subjects viewed words under intentional learning conditions, with each word requiring a decision between two choices based on semantic criteria (i.e. living/non-living). Memory for these words was later assessed in a recognition test, allowing ERP activity elicited by study words to be analyzed by subsequent memory performance. The difference due to memory effect (Dm) was defined as a difference in ERPs, collected during the study procedure, between subsequently remembered and forgotten words. Classic Dm effects were observed in both groups, in that recognized words generated greater ERP amplitudes than non-recognized words. The amplitude of the Dm effect was greater in younger than in older participants. Moreover, when memory performance was equal in both groups, their electric distribution over the scalp differed, suggesting that different neural networks underpinned episodic encoding in younger and older participants. Although frontal lobe activity was observed in both groups, the activity in the right parietal area was greater in younger subjects, whereas strong activity was observed in the left parieto-occipital areas of elderly individuals. Finally, we observed a strong correlation between performance in the test and neural activity during encoding. These findings indicated that recognition memory in older adults was linked to neural activity in the left parietal hemisphere, whereas performance was positively correlated with right parietal activity in younger adults. In conclusion, when older subjects performed equally well as young subjects, encoding processes appeared to be shifted from the right to left hemisphere, suggesting a compensatory mechanism with age.

Disclosures: **D. Tromp:** None. **A. Dufour:** None. **T. Pebayle:** None. **O. Despres:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NEXT program (LZ001)

KAKENHI (25245069)

Title: Projecting myself onto pictures: Effects of self-related processes on successful encoding activations

Authors: *K. NORIMOTO, Y. SHIGEMUNE, T. TSUKIURA;
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Abstract: Regions reflecting the processing of self-related information are different from those reflecting the processing of non-self-related information (Georg et al., 2006). Previous studies have reported that memories encoded by the self-referential process are remembered more accurately than those by the other-referential process (Kuiper & Rogers, 1979), and that the self-related information is represented in the cortical midline structures (CMS: Georg & Felix, 2004). However, evidence regarding how the self-referential operations of encoding by projecting oneself onto target pictures affect encoding-related activations is still unavailable. The current fMRI study investigated this issue. In this study, we employed 32 healthy young participants (15 females, mean age: 21.4, SD: 2.0). During encoding with fMRI, in which participants were not instructed to be tested their memories in the subsequent retrieval, participants were required to view target pictures by two ways. In one way (Self), participants viewed pictures by projecting themselves onto a real scene of the pictures, whereas in the other way (Other), target pictures were seen by a third person's view such as TV shows or newspapers. During retrieval, participants were required to recognize whether the pictures were old or new with high and low levels of confidence. Thus, encoding trials were categorized into subsequent hits with high confidence (HH), subsequent hits with low confidence (HL), and subsequent misses with high and low confidence (M) in each of Self and Other. Behavioral results showed significantly better remembering of pictures encoded in Self than those in Other, but the memory enhancement was found only in HH but not in HL. In fMRI results, which were identified by a 2 (Viewpoint: Self, Other) x 3 (Memory: HH, HL, M) flexible factorial model, the thalamic nuclei showed greater activations in Self than in Other (main effect of Viewpoint), and activations in the medial temporal lobe (MTL: parahippocampal gyrus and hippocampus) and fusiform regions were greater in HH than in HL or M (main effect of Memory). In addition, a medial part of the superior frontal gyrus and a lateral part of the inferior frontal gyrus showed a significant interaction between Viewpoint and Memory factors, in which these regions showed greater activations during HH than HL or M only in Self but not in Other. These findings suggest that the memory enhancement by projecting oneself onto target pictures could be associated with a network including the self-related thalamic region, the memory-related MTL regions, and the medial and lateral prefrontal cortices related to the regulation of self-related information.

Disclosures: K. Norimoto: None. **Y. Shigemune:** None. **T. Tsukiura:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH07458

Title: Modulation of functional connectivity between left inferior frontal gyrus and posterior cortical regions as a function of successful vs. unsuccessful source encoding

Authors: *J. X. WONG, D. R. KING, E. D. HORNE, M. D. RUGG;
Ctr. for Vital Longevity, Univ. of Texas At Dallas, Dallas, TX

Abstract: In the present experiment, the neural correlates of the successful encoding of item-item and item-context associations, as identified with fMRI, were directly compared. While undergoing scanning, subjects (N=20) viewed a series of study trials, each containing the sequential presentation of a picture and a concrete word (presentation duration of each item = 1000 ms, inter-stimulus interval = 500 ms) that required a relational judgment (which of the denoted objects was the smaller). The picture was presented either to the left or right of fixation, whereas the word appeared in central vision. Memory for the study trials was tested outside of the scanner approximately 20 min later. Test items comprised a mixture of studied and unstudied pictures. Each test trial began with a recognition memory test for the presented picture. For pictures that were judged old, subjects were then required either to retrieve the word with which the picture had been associated at study, or the location (left vs. right) of the picture's presentation. In a previous analysis we reported that successful encoding of item-item and item-context associations were associated with enhanced study activity ('subsequent memory effects') in distinct anatomical regions. In particular, the left inferior frontal gyrus (LIFG), was uniquely sensitive to the encoding of item-item associations, suggesting that this region plays a selective role in the encoding of inter-item, but not item-context, associations. Here, we employed psychophysiological interaction analyses (PPI) to identify changes in functional connectivity associated with successful inter-item and item-context encoding. When the LIFG was employed as a seed region, its connectivity with several other cortical regions was enhanced during study trials that went on to attract correct rather than incorrect source judgments. One of these regions - the right lateral occipital complex - had previously been demonstrated to manifest a selective subsequent source memory effect. The findings suggest that our prior conclusion that the LIFG plays a more important role in the encoding of item-item than item-context associations requires qualification: whereas mean signal change in this region is selective for the successful encoding

of inter-item associations, changes in connectivity implicate the region in the encoding of both inter-item and item-context associations.

Disclosures: **J.X. Wong:** None. **D.R. King:** None. **M.D. Rugg:** None. **E.D. Horne:** None.

Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 741.11/RR3

Topic: F.01. Human Cognition and Behavior

Support: R01MH074528

Title: Neural correlates during memory encoding associated with resistance to retroactive interference

Authors: ***J. D. KOEN**, M. D. RUGG;
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Abstract: An extensive literature has demonstrated that learning a new event can induce retroactive interference that results in forgetting a previously encountered event. However, little is known about the neural correlates during encoding that predict resistance to retroactive interference. To address this issue, participants underwent fMRI while encoding words in a paradigm designed to induce retroactive interference. Participants performed one of four tasks for each word during encoding. Words in the Interference condition were presented twice whereas words in the Control condition were presented only once. The task performed during the first presentation of an Interference word always differed from the task performed during its second presentation. Following encoding, participants completed a source memory test where they were required to recall the tasks that were performed with each studied word. The behavioral results demonstrated a significant retroactive interference effect such that source memory for the task associated with the first presentation of an Interference word was significantly lower than source memory for words in the Control condition. We probed the fMRI data for neural activity associated with resistance to retroactive interference by contrasting the magnitude of the subsequent memory effect for the first task as function of accuracy of source memory for the second task. The idea behind this contrast is that retroactive interference will be more likely to occur when the second task is remembered, and differences in encoding-related activity likely reflect processes conferring resistance to retroactive interference. Activity in

bilateral frontal operculum/insula, right inferior frontal gyrus, and the anterior medial temporal cortex/hippocampus was associated with resistance to retroactive interference. These regions showed larger subsequent memory effects for the first task (i.e., remembered > forgotten) when the second task was also remembered than when the second task was forgotten. Subsequent source memory effects that were independent of the strength of retroactive interference were identified in left inferior temporal and frontal gyri, right precuneus, and right parieto-temporal cortex. These results suggest that engagement of regions implicated in cognitive control and episodic memory support the formation of memory traces resistant to subsequent retroactive interference.

Disclosures: J.D. Koen: None. **M.D. Rugg:** None.

Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 741.12/RR4

Topic: F.01. Human Cognition and Behavior

Support: NSERC RGPIN 8347

Title: Prior knowledge effects on post-encoding brain connectivity

Authors: *Z. LIU[1,2], C. GRADY[2,3,4], M. MOSCOVITCH[2,3];
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Abstract: Post-learning brain activity has been shown to reflect off-line memory processing, possibly related to memory consolidation processes. Recent studies also found that prior knowledge may facilitate memory consolidation. However, how prior knowledge can affect post-encoding brain activity has not been investigated thoroughly. In this fMRI study, we used an explicit encoding task, in which participants associated novel houses with famous or nonfamous faces, to investigate how associative encoding tasks with or without prior knowledge (i.e., famous vs. nonfamous condition) differentially affected post-encoding brain connectivity measured during rest periods. Using selected ROIs (based on encoding brain activity), we found that the post-encoding functional connectivity between the right hippocampus (HPC) and left fusiform face area (FFA) was stronger following encoding of associations with famous than non-

famous faces. Functional connectivity between the left HPC and the right FFA, between the left anterior temporal pole region (aTPL) with the parahippocampal place area (PPA) and left FFA, and between the right aTPL and the left PPA predicted later associative face-house memory, but not item memory, only in the famous condition. These results indicate that when prior knowledge is involved, the HPC and aTPL, which support prior episodic and semantic memories, respectively, continue to interact with the posterior perceptual brain regions (e.g., the PPA and FFA) during the post-encoding rest to facilitate off-line processing of the newly formed memory.

Disclosures: Z. Liu: None. **C. Grady:** None. **M. Moscovitch:** None.

Poster

741. Human Long-Term Memory: Encoding

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Program#/Poster#: 741.13/RR5

Topic: F.01. Human Cognition and Behavior

Support: NINDS Intramural Research Program

Title: Human intracranial EEG reveals distinct a time course of cortical activation during proactive versus retroactive attention-enhanced memorization

Authors: *J. H. WITTIG, JR[1], R. ELLENBOGEN[2], S. INATI[2], K. ZAGHLOUL[2]; [1]NINDS, BETHESDA, MD; [2]NINDS, Bethesda, MD

Abstract: Humans are better at remembering a stimulus when they focus attention either before (proactively) or after (retroactively) stimulus presentation. fMRI studies have revealed that activation patterns during proactive and retroactive attention-enhanced memorization are similar, which has been interpreted to mean they share a common neural mechanism. Here we examine the neurophysiological correlates of proactive and retroactive attention-enhanced memory in human participants undergoing intracranial EEG monitoring for pharmacologically intractable epilepsy. We asked the participants to view a list of serially presented words, and to remember just those words that were immediately preceded or followed by a visual cue. Recognition memory of both cued and non-cued words was assessed approximately 30 seconds after list presentation. In five participants, recognition memory performance matched three criteria: cued word performance was significantly better than non-cued word performance; non-cued word performance was significantly better than chance; and performance among cued words was

indistinguishable whether the cue had been proactive or retroactive. We examined the time course of low (2-32 Hz) and high (32-400 Hz) frequency power during encoding, and compared spectral power between successfully remembered cued versus non-cued words. Across five participants with a total of 374 implanted electrodes, low frequency power significantly decreased and high frequency power significantly increased during memorization of cued words relative to non-cued words. The time course of power changes differed for proactive and retroactive cues: proactive cues elicited power changes (low and high frequency) during word presentation, and during the maintenance period that preceded the next word in the list; retroactive cues only elicited power changes (low and high frequency) once the attention cue had appeared during the maintenance period. The distinct time course of power changes for proactive and retroactive cues raises the possibility that these two forms of attention-enhanced memory are supported by distinct neural mechanisms.

Disclosures: J.H. Wittig: None. **R. Ellenbogen:** None. **S. Inati:** None. **K. Zaghloul:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NIH Intramural Program

Title: Reinstatement of distributed spatiotemporal patterns of oscillatory power during associative memory recall

Authors: R. B. YAFFE[1], M. S. D. KERR[1], S. DAMERA[2], S. V. SARMA[1], S. K. INATI[2], *K. A. ZAGHLOUL[3];
[1]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [3]Surgical Neurol. Br., Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: Models of episodic memory suggest that neural activity present during encoding is reinstated during successful retrieval. Recent fMRI studies provide empiric support for neural reinstatement by using activity during retrieval to classify distinct encoding categories, but do not directly examine trial specific reinstatement with high temporal precision. Here, we used intracranial recordings to directly examine the spatiotemporal extent of neural reinstatement

when encoding and retrieving individual stimuli as 32 participants with electrodes placed for seizure monitoring engaged in a paired associates episodic verbal memory task. We found significantly greater reinstatement of the distributed pattern of oscillatory power across multiple spatial locations and multiple frequency bands during trials that exhibited successful encoding and retrieval compared to incorrect trials. Consistent with theoretical and experimental work, we found that reinstatement was modulated by memory strength and by temporal context. We examined individual neural features and found that reinstatement was largely mediated by theta and high gamma frequencies in the temporal lobes. Leveraging the high temporal precision afforded by intracranial recordings, we found that high gamma activity associated with reinstatement preceded theta activity during both encoding and retrieval, but during retrieval the difference in timing between frequency bands was compressed.

Disclosures: **R.B. Yaffe:** None. **M.S.D. Kerr:** None. **S. Damera:** None. **S.V. Sarma:** None. **S.K. Inati:** None. **K.A. Zaghloul:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: SNSF P2LAP3-151771 to AT

SNSF 320030-149982 to MMM

Swiss Brain League 2014 Research Prize to MMM

Title: Multisensory context portends object memory

Authors: *A. THELEN[1,2], P. J. MATUSZ[2], M. M. MURRAY[2];
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Abstract: It is well established that multisensory processes facilitate perception of currently-presented stimuli. Multisensory processes can likewise enhance later object recognition; memories for objects originally encountered in a multisensory context can be more robust than those for objects encountered in an exclusively visual or auditory context [Thelen and Murray, 2013]. These observations upturn one of the most fundamental assumptions about memory

performance, i.e., that memory performance is best when encoding and recognition contexts remain constant. Here we used electrical neuroimaging of event-related potentials (ERPs) to provide the first evidence for a direct link between multisensory brain activity at one point in time and object discrimination abilities at a later point in time. Across two experiments we show that brain responses to multisensory stimuli within parietal cortices differentiated between individuals who would show a benefit and those showing a cost during later object discrimination. These effects were observed despite the multisensory information being presented only once and being task-irrelevant. Importantly, perceptual preferences between the groups could not explain the results. We provide a critical novel insight into the advantages associated with multisensory interactions, by showing they are not limited to the processing of current stimuli, but likewise encompass the ability to determine the benefit of one's memories for object recognition in later, unisensory contexts.

Disclosures: **A. Thelen:** None. **P.J. Matusz:** None. **M.M. Murray:** None.

Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 741.16/RR8

Topic: F.01. Human Cognition and Behavior

Title: Developing support vector machine classification of associative memory for real-time fMRI

Authors: ***H. DESHPANDE**[1,2], A. EKLUND[1], J. LISINSKI[1], C. MUELLER[1], B. KING-CASAS[1,2], S. LACONTE[1,2];
[1]Virginia Tech. Carilion Res. Inst., Roanoke, VA; [2]Sch. of Biomed. Engin. and Sci., Virginia Tech., Blacksburg, VA

Abstract: Introduction: Classification-based real-time functional magnetic resonance imaging (rtfMRI) can decode subjects' brain activity to control an fMRI stimulus, potentially enabling therapeutic neurofeedback. Based on the success of region-of-interest-based rtfMRI [1], we are developing a support vector machine (SVM)-based rtfMRI system that can explore various facets of learning and memory. Reported here are results from a verbal paired associate memory study. Methods: fMRI data were collected on a 3T MRI (TR/TE = 2000/30 ms, 64×64 acquisition matrix, 3.6-mm slice thickness, 33 slices, 220-mm field of view). Participants first performed a memorization task with 70 pseudo-word pairs [2] followed by a multiple choice recognition task.

They performed 2 runs of each task in the scanner, with identical word pairs presented in random orders and with different multiple choice distractors. Preprocessing and SVM classification were performed using AFNI [3] and 3dsvm [4]. The memorization data were "labeled" with their subsequent recognition performance to train and test an SVM. By alternatively using one memorization run as training data and the other as test data, we were able to obtain cross-validated classification accuracies. Results: The subjects' recognition performance on the first and second recognition tasks was 40% and 50%, respectively (chance = 25%). The average SVM accuracy was 53% (31% and 22% were correctly classified as learned and not learned, respectively. While 19% and 27% were incorrectly predicted as learned and not learned, respectively). Fig. 1 shows examples of actual SVM output during correct fMRI classifications. Conclusions: Our results support the possibility of designing rtfMRI experiments to develop refined learning strategies as well as to neurofeedback-based rehabilitation for memory deficits after stroke/head injuries. References: [1] Gabrieli, et al. 2012. [2] Medler and Binder, 2005. [3] Cox, 1996. [4] LaConte, et al. 2005.

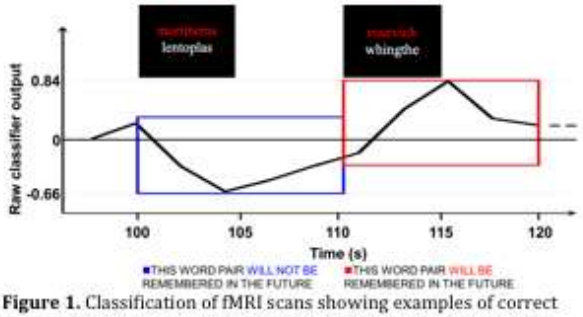

Figure 1. Classification of fMRI scans showing examples of correct classification of a "not-learned" and "learned" set of pseudo-word pairs.

Disclosures: H. Deshpande: None. **A. Eklund:** None. **J. Lisinski:** None. **C. Mueller:** None. **B. King-Casas:** None. **S. LaConte:** None.

Poster

741. Human Long-Term Memory: Encoding

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Program#/Poster#: 741.17/RR9

Topic: F.01. Human Cognition and Behavior

Support: The John Templeton Foundation grant #36751

Title: Imagine the future! How does episodic simulation enhance prospective memory?

Authors: *I. MOMENNEJAD, J. D. COHEN, K. A. NORMAN;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Imagining task-related future episodes enhances prospective memory (PM) (Brewer et al. 2011). One hypothesis is that episodic simulation recruits mechanisms that spontaneously bring prospective intentions to mind at the right time (Einstein and McDaniel, 2000). However, the neural mechanisms underlying the role of episodic future simulation in prospective memory have not yet been established. We suggest that (a) spontaneous retrieval processes in PM rely on episodic memory (Cohen and O'Reilly, 1996), and (b) imagining task-related future episodes (episodic "preplay") increases the odds that the memory trace laid down during intention-formation will match the participant's mental state when the PM target appears. This increase in study-test match should (in turn) increase the likelihood that the PM target will trigger spontaneous retrieval of the PM intention. This view predicts that neural evidence for accurate episodic future simulation (i.e., preplay of features that match the PM target) would correlate with prospective memory success. To test this prediction, we used multi-voxel pattern analysis (MVPA) of functional magnetic resonance imaging (fMRI) data from a non-focal prospective memory paradigm. Participants were instructed to perform a numerical parity or magnitude task at fixation (ongoing task). while faces and scenes appeared peripherally on the left or right side of the screen. Participants were instructed to respond to a specific peripheral face or scene image (non-focal PM target) when it appeared on a specific side of the screen (left or right). In each trial, participants were informed about their future target (the identity of the image, and the side of the screen on which it would appear) and the ongoing task they would be performing when it appeared. After instruction, participants were asked to mentally simulate the future target and rate the quality of their imagery (20 s) before the start of the task. Behaviorally, we found a positive correlation between the ratings of imagery quality and PM hit rate. In order to test whether neural evidence for future simulation correlated with PM success, we trained a classifier on a functional localizer with images of faces and scenes (appearing on the left or right, during an ongoing parity or magnitude task), and we used these parameters to decode what the subjects "imagined" during the simulation period, along the following four dimensions: left/right, image category, specific image identity, and ongoing task (parity/magnitude). We will present the results of analyses exploring whether MVPA evidence of preplay during the simulation period predicts PM success.

Disclosures: I. Momennejad: None. **J.D. Cohen:** None. **K.A. Norman:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: Economic and Social Research Council

Deutscher Akademischer Austauschdienst

Title: Using functional magnetic resonance imaging (fMRI) to investigate the neural bases of cognitive contributions to emotional enhancement of memory (EEM)

Authors: *G. E. BARNACLE[1], D. TALMI[1], T. SOMMER[2];
[1]Dept. of Psychology, Univ. of Manchester, Manchester, United Kingdom; [2]Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Explanations of EEM commonly focus on neural processes at consolidation; however behavioural evidence suggests that cognitive factors at encoding may also contribute. The cognitive factor 'distinctiveness processing' was operationalised by comparing mixed lists (emotional and neutral items intermixed; evoking distinctiveness processing) to pure lists (all same valence items; no distinctiveness processing). Twenty-two healthy female participants intentionally encoded sixteen lists (eight of each type) containing fourteen colour pictures. Each encoding list was followed by a sixty second distractor task, after which a three minute free recall task ensued. We used fMRI to investigate how distinctiveness processing differentially affected the encoding of emotional and neutral pictures whilst controlling within-set semantic relatedness (noted as a significant contributor to EEM) using a subsequent memory paradigm. Behavioural results replicated our previous findings: EEM was eliminated when comparing memory for pure lists of emotional and neutral stimuli, but a significant EEM was identified in mixed lists. Additionally, in keeping with the classical 'list strength' effect, memory for neutral items was higher in pure lists and memory for negative items higher in mixed lists, although only the latter effect reached significance. We found that EEM in mixed lists was supported by activity in inferior parietal lobule (IPL) and anterior cingulate cortex (ACC). The ventromedial prefrontal cortex (vmPFC) was activated during encoding of mixed lists but not pure. Bilateral (para)hippocampal regions were associated with neutral memory in pure but not mixed lists, and memory for negative items in mixed but not pure lists was associated with amygdala activity (AMY). These findings extend a substantial previous literature regarding the EEM by acknowledging the contribution of distinctiveness processing, and provide insight on the neural mechanisms that allow negative items to stand out at encoding which subsequently affects memory performance.

Disclosures: G.E. Barnacle: None. **D. Talmi:** None. **T. Sommer:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NIA grant R01AG026308

Title: An ERP investigation of the formation of unitized memory representations of arbitrary word pairs

Authors: *H. D. LUCAS[1], R. HUBBARD[2,1], K. ABUSAGER[1], K. D. FEDERMEIER[2,1]; [1]Beckman Inst. for Advanced Sci. and Technol., [2]Dept. of Psychology, Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: When meaningful stimuli such as words are encountered in groups or pairs (e.g., "elephant-ferry"), they can be processed either separately or as a unitized semantic concept ("an elephant ferry"). Prior research suggests that memory for unitized associations is supported by different mechanisms than is memory for non-unitized associations, with the former showing less hippocampal dependence and smaller age-related deficits. However, little is known about the neurocognitive mechanisms that promote unitization. In the present study, we recorded ERPs while participants attempted to construct coherent (unitized) definitions for pairs of sequentially presented, unrelated nouns. To examine the role of imagery in unitization, we included both concrete-concrete (e.g., "blanket-clay") and abstract-concrete noun pairs ("irony-shield"). Drawing on research in compositional language processing (Huang & Federmeier, 2010, 2012), we hypothesized that the concreteness of the first noun would moderate the ease with which it could be semantically integrated with the second noun, resulting in facilitated unitization for concrete-concrete relative to abstract-concrete pairs. Consistent with these predictions, concrete-concrete pairs received higher ease-of-unitization ratings from participants, and were also better remembered on a subsequent associative memory test. Moreover, ERPs recorded during the second noun in each pair differed as a function of the concreteness of the first noun. These compositional concreteness effects included more positive N400 potentials linked to predictive processing and more negative late frontal ERPs linked to imagery for second nouns preceded by concrete relative to abstract first nouns. Importantly, when participants studied the same noun pairs while performing a task that did not encourage unitization, the late frontal compositional concreteness effect disappeared, and associative memory performance worsened. These data support a role of predictive language processing and compositional imagery in constructing unitized semantic representations of word pairs. Moreover, previous research has found that

older adults show reduced compositional concreteness effects during natural language tasks, such as when processing coherent adjective-noun pairs (Huang & Federmeier, 2012). Thus, the present findings raise the possibility that the effectiveness of unitization as a mnemonic strategy may depend in part on the integrity of basic integrative language-processing mechanisms.

Disclosures: H.D. Lucas: None. **R. Hubbard:** None. **K. Abusager:** None. **K.D. Federmeier:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NWO Grant 452-09-007

Title: Reacting to novelty: Effects on learning, and the role of expectations

Authors: *M. MEETER, J. SCHOMAKER, M. RANGEL GOMEZ;
Dept of Cognitive Psychology, VU Univ. Amsterdam, Amsterdam, Netherlands

Abstract: In many brain regions, signatures can be found of the brain's novelty processing mechanisms. At the neural level, single cell recordings in primate inferior temporal cortex have shown much stronger neural firing to novel as compared to familiar stimuli. In humans, electrical recordings on the scalp show evoked response potentials (ERPs) that are linked to novelty processing, the anterior N2, and the P3a. We used these components to investigate two questions. First we looked at whether novelty responses are truly a function of novelty, or of deviation from expectations. A novel stimulus is by definition somewhat unexpected, since the brain cannot expect stimuli that it has never experienced. However, we manipulated the gradation of unexpectedness using cues that foretold when novel stimuli were likely. We found that being able to predict when novel stimuli are presented reduces novelty-associated ERP components elicited by these stimuli, but not by much. This suggests a role for the violation of expectations in novelty processing, but not a large one. Second, we looked at whether experiencing novelty aids in episodic learning. This is generally thought to be the case, but evidence for it is not thick on the ground. In several experiments, we found that novelty does generate dynamics that foster learning on a time scale of minutes, but not on a time scale of seconds.

Disclosures: **M. Meeter:** None. **J. Schomaker:** None. **M. Rangel Gomez:** None.

Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

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Program#/Poster#: 741.21/RR13

Topic: F.01. Human Cognition and Behavior

Title: Multivariate pattern analysis on subjective processing of temporal memory in hippocampus

Authors: ***S. YI**[1], N.-Y. SHIN[2], S. HAN[1], S.-K. LEE[2];
[1]Dept. of Psychology, Yonsei Univ., Seoul, Korea, Republic of; [2]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Memory and its contextual features have been topics of great interest in field of psychology and neuroscience, but neural underpinning of how temporal context is processed remains unclear. Most of researches on temporal context of memory have based their analysis on the actual chronological order of processed information rather than subjective processing of temporal information. We focused on the subjective processing of temporal context involving neural representation in hippocampus, region known to carry temporally evolving context representation. Our fMRI experiment was consisted of 2 cycles of a consecutive encoding and retrieval session, and each cycle was in the same manner except the assigned task in encoding sessions differed in the involved level-of-processing. In encoding session of the first cycle, a participant was given pictures of objects and decided whether the color of the object was close to green or red (Color decision task), while chose whether the object felt pleasant or unpleasant (Emotion decision task) in the second cycle. In retrieval session, the pictures of previously seen objects were presented and the participant indicated perceived temporal positions of the objects on a wide bar. In analysis of the results, if the item was recalled to be positioned before its actual position in the retrieval session, then the response was called the have ‘backward’ temporal bias, and ‘forward’ bias in the other way around. Both bias conditions were divided into 2 groups; responses with biases of magnitude over the half-line of highest possible was assigned into strong groups and the other halves into weak bias groups. In our analysis to discriminate representational patterns of hippocampal activities in subjective temporal processing of information, Multivariate Pattern Analysis (MVPA) was implemented with linear Support Vector Machine (SVM) classifier. While prediction accuracies comparing two encoding tasks

were not different in both encoding and retrieval sessions, comparisons between strong biases (backward vs. forward) and between weak biases reached across task average of 94.2% and 62.2% respectively in subsequent analysis of encoding sessions, indicated that more discriminable neural patterns were induced by stronger biases. Analyses of retrieval session resulted accuracies indifferent from chance level. We showed that neuronal processing of information bound to be temporally biased in opposite directions are differentiable and it can be assumed that subjective temporal perception of memory is based on information processing in encoding stage rather than retrieval of encoded information, from the results of our analyses.

Disclosures: **S. Yi:** None. **N. Shin:** None. **S. Han:** None. **S. Lee:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R00MH083945

Title: Dissociable neural correlates of unitization and relational association at encoding

Authors: ***H.-W. TU**, V. L. BRAYMAN, R. A. DIANA;
Psychology Dept., Virginia Tech., Blacksburg, VA

Abstract: During an event, context details are typically encoded through arbitrary associations with an item and are strongly supported by recollection at retrieval. Recent studies have suggested that source recognition could be a familiarity-based process when the context is "unitized" as a feature of the item in a single mental image created at encoding. However, most investigations on the effect of unitization have focused on the contribution of familiarity and recollection during retrieval. It is still unclear whether unitization during encoding initiates distinct neural processes in contrast to relational associations during encoding. To fill this research gap, we used event-related potentials to monitor immediate brain activities at encoding while participants were instructed to simply associate or strategically unitize the study word and corresponding color information. Results showed that unitized encoding was associated with a frontally distributed positivity from 500 to 1250 ms, whereas relational encoding was associated with a parieto-occipitally distributed positivity from 750 to 1500 ms. Furthermore, receiver operating characteristic curves indicated a significantly higher contribution of familiarity to

source recognition when the context detail was unitized at encoding than when it was not. Our findings showed that different encoding strategies elicit unique neural correlates during specific time course, which may change the mnemonic processing for subsequent source recognition.

Disclosures: H. Tu: None. **V.L. Brayman:** None. **R.A. Diana:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: Postdoctoral Research grant by Basque Country Government

Title: Resting state connectivity related with retrieval practice

Authors: *E. MARIN-GARCIA[1,2,3], A. T. MATTFELD[3], J. D. E. GABRIELI[3];
[1]Basque Ctr. on Cognition, Brain and Language, San Sebastian, Spain; [2]Ikerbasque, Bilbao, Spain; [3]Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Retrieval practice compared to study alone increases memory performance, which is known as the testing effect. Correlations in resting state activity can provide insight into the function of neural systems, detecting networks engaged during cognitive tasks. The main goal of this study was to examine inter group differences in resting-state connectivity in relation to the use of different tasks during training: retrieval practice or study alone. Participants were instructed to learn Swahili-English vocabulary word pairs during training outside of the scanner. They were assigned randomly to the Study group, which only studied the word pairs, or to the Test group, which both studied and tested the word pairs. After a week delay, we measured resting state functional connectivity in areas previously associated with test-potentiated learning such as the hippocampus (Wing et al. 2013). Immediately after the resting state scan, we examined memory performance with a cued recall test. The Test group showed increased functional connectivity between the bilateral hippocampus and premotor areas and somatosensory association cortex compared to Study group. In contrast, the Study group showed greater functional connectivity between left hippocampus and left parietal compared to Test group. Behaviorally, the testing effect was confirmed; Test group had significantly greater memory performance than the Study group. Differences in functional connectivity between groups reflect the engagement of distinct networks related to different training procedures

performed a week earlier. The functional connectivity between memory related regions and motor regions perhaps related to speech production in the Test group is consistent with our task based fMRI results. The development of this link as evinced by increased functional coupling at rest may confer this memory benefit. These results suggest that subtle training related differences lead to long lasting brain differences facilitating the communication between networks of regions important for learning and memory.

Disclosures: E. Marin-Garcia: None. **A.T. Mattfeld:** None. **J.D.E. Gabrieli:** None.

Poster

741. Human Long-Term Memory: Encoding

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Topic: F.01. Human Cognition and Behavior

Support: John Templeton Foundation

Title: Neural evidence for a context-change account of list-method directed forgetting

Authors: *K. NORMAN[1], J. R. MANNING[1], J. A. WILLIAMS[1], L. R. PILOTO[1], J. C. HULBERT[1], B. ABUSHANAB[2], L. SAHAKYAN[2];
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Abstract: How do people deliberately forget a just-experienced event? This issue has been studied in the cognitive psychology literature using the list-method directed forgetting paradigm. In this paradigm, participants study a list of words (list A) followed by a second list of words (list B), followed by a memory test for list A or list B. Between list A and list B, participants are given a cue to either remember or forget list A; the standard behavioral finding is that instructions to forget list A lead to impaired memory for list A and improved memory for list B. According to the context-change hypothesis of directed forgetting, participants respond to the forget cue instruction by deliberately shifting their mental context (Sahakyan & Kelley, 2002); if participants encode list B items in a different mental context from list A items, and the list B context is differentially accessible at test, this should improve list B recall and impair list A recall. While this context-change theory has gained traction in the cognitive psychology literature, there is (as of yet) no concrete neural evidence that the theory is correct. To address this shortcoming, we ran a study where we used multivariate pattern classification of fMRI data to measure participants' context shift. The study used a standard directed forgetting paradigm,

with one key change: We interposed images of scenes between the list A (but not list B) words. The scenes presented during list A study played a role analogous to radioisotope tracers used in PET scanning: Scene-related processing is highly visible in fMRI; consequently, "injecting" scene-related processing into the list A context (but not the list B context) made it possible for us to track the persistence (or lack thereof) of the list A context over the course of the rest of the experiment. We hypothesized that, if participants "flushed out" their list A mental context in response to forget (but not remember) cues, we would see a differential decrease in scene activity in response to forget (vs. remember) cues. We tested this prediction using a classifier trained to detect scene processing. As predicted by the context-change hypothesis, scene classifier evidence decreased more sharply after forget (vs. remember) cues. We also found that (on a run-by-run basis) frontal activity elicited by the forget/remember cue predicted the size of the drop in scene evidence, suggesting that these frontal regions play a role in flushing out the list A context. Additional analyses will explore the relationship between these neural effects (the drop in scene evidence and the corresponding frontal activations) and the effects of remember/forget cues on behavioral recall performance.

Disclosures: K. Norman: None. **J.R. Manning:** None. **J.A. Williams:** None. **L.R. Piloto:** None. **J.C. Hulbert:** None. **B. Abushanab:** None. **L. Sahakyan:** None.

Poster

741. Human Long-Term Memory: Encoding

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 741.25/RR17

Topic: F.02. Animal Cognition and Behavior

Support: Ontario MRI ERA

Canadian Foundation for Innovation

Alfred P. Sloan Foundation

NSERC

NSERC-CREATE VSA Program

Krembil Foundation

Title: Visual exploration states during memory encoding in macaque hippocampus

Authors: *R. MONTEFUSCO-SIEGMUND[1,4], T. K. LEONARD[2,4], K. L. HOFFMAN[2,3,4]; [2]Psychology, [3]Biol., [1]York Univ., Toronto, ON, Canada; [4]Ctr. for Vision Res., Toronto, ON, Canada

Abstract: The hippocampus is critical for forming episodic memories and for spatial exploration. Hippocampal neurons show oscillatory activity in the theta band (4-12 Hz) during repetitive exploratory behaviors in mammals, including whisking, sniffing, echolocation, and more recently 'scanning' breaks during locomotion. In primates, the predominant modality of exploration is vision, with 3-5 Hz saccadic eye movements punctuating sampling that occurs during fixations. The spatial distribution of fixations, however, is not even. Bouts of local exploration are characterized by small saccades and long fixations, and global exploration with opposite characteristics. Here, we sought to address whether hippocampal activity varies with these visual exploratory states. To investigate this, we examined local field potential activity in the macaque hippocampus during bouts of local and global exploration in a change detection task, where animals were required to find changing items ('targets') within flickering images of natural scenes, yielding faster search times for remembered targets than for forgotten ones on repeated trials (Chau et al., 2011). In exploratory trials where search times were greater than 20 seconds, we defined local/global episodes based on the number of consecutive saccade lengths under/over the 33rd/66th percentiles, respectively, and which fell under/over a cumulative distance threshold for each cluster of consecutive saccades. Local exploration bouts produced greater power in the theta/alpha (6-13 Hz) and beta (19-24 Hz) bands compared to that of global bouts. The different exploratory states were therefore associated with changes in hippocampal oscillations. Specifically, low-frequency theta/alpha activity associated with local search may be analogous to the active exploratory behaviors of other mammals. This may be useful for encoding local features of a visual scene, due to theta-band plasticity which may underlie memory formation.

Disclosures: R. Montefusco-Siegmund: None. **T.K. Leonard:** None. **K.L. Hoffman:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.01/RR18

Topic: F.01. Human Cognition and Behavior

Title: Decrease in depressive symptoms after ketamine is associated with change in amygdala response to implicit positive emotion

Authors: *J. E. SZCZEPANIK[1,2], M. FUREY[1], N. ALLISON[1], C. ZARATE[1];
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Abstract: The amygdala is implicated in emotion processing, and responses are modulated by valence, attentional demands and mood. Amygdala activity during emotional tasks is altered in Major Depression (MDD) and changes after successful antidepressant medication or cognitive therapy. We investigated the relationship between neural activity in the amygdala during processing of emotional faces and antidepressant response to the rapidly acting NMDA antagonist ketamine. 16 unmedicated MDD patients(7 m) participated in a placebo-controlled, crossover trial of i.v. ketamine (.5mg/kg). Depressive symptoms were assessed with Montgomery-Asberg Depression Rating Scale (MADRS). BOLD signal was measured using fMRI pre and post infusions. Subjects responded to emotional faces under explicit (judge emotion as positive or negative) and implicit (judge gender) conditions. A 5 mm center of mass sphere was placed in the left and right amygdala where change in response to masked emotional faces correlated with treatment outcome (Victor, 2010). BOLD signal for voxels falling within each sphere was averaged for each subject/task condition. Correlations between pre-infusion BOLD and subsequent % change in MADRS post ketamine were calculated. Change in BOLD response pre-vs-post ketamine and placebo also was correlated with the magnitude of antidepressant effect. Treatment response to ketamine correlated with pre-treatment BOLD response in the right amygdala to the explicit positive condition ($r = +.6$, $p < .025$) and the implicit positive condition($r = -.57$, $p < .025$). No correlation was found with placebo response. The BOLD signal change from pre to post-ketamine in the right amygdala during the implicit positive condition also correlated with the andtidepressant effect ($r = .7$, $p = 0.005$). We found that the pre-treatment happy face processing in both implicit and explicit conditions predicted clinical response to ketamine, while for change in BOLD response (pre versus post-ketamine), only the implicit happy condition correlated with the magnitude of the antidepressant effect. This finding is compatible with previous results showing that neuronally based emotion responses in depression shift after treatment, and our findings indicate that amygdala processing of positively valanced stimuli is associated with clinical improvements. Our results support the hypothesis that amygdala responses to emotional stimuli may constitute a biomarker of treatment response. Further study will examine amygdala role in explicit and implicit processing of emotion before and after ketamine in healthy individuals.

Disclosures: J.E. Szczepanik: None. **M. Furey:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds use patent for scopolamine. **N. Allison:** None. **C. Zarate:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds use patent for ketamine.

Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.02/RR19

Topic: F.01. Human Cognition and Behavior

Support: NICHD Grant 0691780

Title: Activation in the midcingulate cortex supports normative developmental changes in separation anxiety

Authors: *K. LONG, J. A. SILVERS, K. N. OCHSNER;
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Abstract: The formation of a secure attachment to one's primary caregiver is a critical feature of early childhood development. As such, it is normal for children to exhibit separation anxiety (SA)--fear, distress, or worry when anticipating or experiencing separation from an attachment figure. While much clinical work has investigated a pathological variant of SA, separation anxiety disorder (SAD), only a handful of studies have focused on normative developmental changes in SA and few have involved neuroimaging data. Given evidence linking SA to maladaptation, higher pain reactivity, and negative affect, it was hypothesized that SA may mediate age-related changes in brain regions involved in affective processing. To test this hypothesis, 62 participants aged 6-17 completed a neuroimaging experiment that involved looking at aversive and neutral social stimuli and provided responses to the Screen for Child Anxiety Related Emotional Disorders (SCARED). Across the sample, age predicted less SA, yet there was also significant inter-individual variability in SA. For aversive trials, on average, age predicted decreased recruitment of the midcingulate cortex (MCC), a brain region commonly implicated in physical and emotional pain processing as well as cognitive control. Yet, children and adolescents high in SA showed elevated MCC activation relative to children and adolescents low in SA. To test whether SA mediates age-related changes in MCC recruitment, a single-level mediation analysis was conducted. Each of the pairwise associations between age, MCC activation, and SA were significant. Critically, the association between age and MCC recruitment was fully mediated by SA such that the relationship between age and MCC activity became non-significant after accounting for SA. This pattern of findings was unique to SA and did not extend to other forms of anxiety. Taken together, this suggests that SA is a critical component of age-related changes in the recruitment of affective processing networks. This

finding has implications for understanding the pathogenesis and developmental course of SAD and other anxiety disorders.

Disclosures: K. Long: None. **J.A. Silvers:** None. **K.N. Ochsner:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

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European Research Council

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Söderberska Stiftelsen

Title: Functional interactions between perceptual and affective body representations in the brain: Implications for eating disorder vulnerability in women

Authors: *C. E. PRESTON, H. EHRSSON;
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Abstract: Negative feelings towards our body can have devastating consequences being associated with eating disorders (EDs) such as anorexia nervosa (AN). However, little is known about the brain mechanisms involved in the development of such negative feelings towards the own body. The current study used multisensory illusions to modulate perceived body shape of healthy participants whilst registering changes in brain activity using functional magnetic resonance imaging (fMRI) and behavioural changes in reported body satisfaction. 32 participants (16F) were recruited. Pre-experiment screening ensured inclusion criteria of no current or previous psychiatric disorders and no clinical levels of ED psychopathology. This also provided

an (non-clinical) ED psychopathology score for each participant. During fMRI participants were given the illusion of owning slim or obese bodies by presenting 3D video images of sex matched bodies filmed from first person perspective and applying synchronous visual tactile-stimulation. Asynchronous visual-tactile stimulation, which does not elicit an illusion, was used as a control for each body type creating a full factorial design. In line with previous studies, subjective experience of the illusion correlated with neural activation in both the premotor (right precentral gyrus, $p<.05$ corrected; left precentral sulcus $p<.01$ corrected) and intraparietal (right intraparietal sulcus, $p<.01$ corrected) cortex. Owning an obese body was more emotionally salient than owning a slim body resulting in significantly lower body satisfaction compared to baseline ($p<.05$). No equivalent effect was found when owning a slim body ($p=.422$). Owning an obese body was also associated with lower activation in the right anterior insula cortex compared to when owing a slim body ($p<.05$ corrected). For the same contrast ED psychopathology correlated with reduced activity in the anterior cingulate cortex (ACC) ($p<.01$ corrected). Relative decreases in ACC activity were also found for females compared to males ($p<.05$ corrected). Psychophysical Interaction Analysis revealed evidence of functional connectivity between intraparietal regions (body perception) and the ACC (emotion) when owning an obese compared to slim body ($p<.01$ corrected). For females the connectivity involved the dorsal/caudal ACC (previously implicated in AN), whereas for males it involved the rostral ACC. These findings provide the first evidence for a direct functional link between neural networks involved with body perception and emotional experience of the body. The findings also provide support for the role of the ACC in the pathophysiolology of EDs particularly in women.

Disclosures: C.E. Preston: None. **H. Ehrsson:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

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Program#/Poster#: 742.04/RR21

Topic: F.01. Human Cognition and Behavior

Support: VIEP-BUAP (No. FLAG/IND14

CONACYT (138663)

Title: Oxidative stress markers in ventromedial prefrontal cortex suicidal people

Authors: *E. BALTAZAR-GAYTAN[1,2], P. AGUILAR ALONSO[3], F. DOLORES GARCIA[4], G. GAYTAN ROMERO[5], F. DE LA CRUZ - LOPEZ[6], G. FLORES[1];
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Abstract: Depression is one of the most common mental disorders, being within the leading causes of disability. Who estimates that 121 million people presented depression in the life, each one of five people will develop depressive symptoms, this number increases with risk factors such as illnesses or stress situations. Another important aspect that has been linked to depression is suicide, the 33% of people that suffer major depression only the 15% finished the suicide. The most vulnerability people are teenagers, this group to be more impulsive in their behavior, and to be subject to parents criticism, they do not show significant changes before the suicide attempt. Furthermore, probability increases when they suffer a loss of something dear or when experience emotions of anger or guilt. The ventromedial prefrontal cortex is involved in the estimation of the long-term consequences of decisions that, participate to the integration of somatic states with key information from the situation itself or stored in memory are taken. Studies of patients with lesions in this region often have impaired social behavior, decision making and emotional processing. Although they have difficulty learning from mistakes, retain their intellectual abilities, intelligence and memory, along with other cognitive functions, at a normal level. However, in its social, labor and economic life, these patients are prone to make decisions and adopt behaviors with negative consequences (suicide). Oxidative stress is a state where there is an imbalance between the processes of pro-oxidant and antioxidant defense system. At cellular level, major depression cause an increase in the production of free radicals or an inefficient antioxidant defense system or both. A disturbance in the antioxidant defense system, which commits antioxidant enzymes such as superoxide dismutase (SOD) due of free radicals, can induce oxidative damage implicated in various neuropsychiatric disorders. Lipid peroxidation is one of the effects of oxidative stress and oxidative deterioration that it acts on the cell membrane lipids, thus producing certain characteristic substances such as malondialdehyde (MDA), the 4-hydroxy-alkenals (4-HDA). Many biochemical factors have been associated with suicide attempts, or have been found postmortem brain studies, so in this paper we analyzed the ventromedial prefrontal cortex of the suicide people, who were aged 14-18 years. We evaluated the SOD activity and nitric oxide levels. Likewise as indicators of lipid peroxidation were determined MDA and 4-HAD levels. (Supported by: VIEP-BUAP grant (No. FLAG/IND14) and CONACYT grant (No. 138663) to G Flores).

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Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.05/RR22

Topic: F.01. Human Cognition and Behavior

Support: Dutch Ministry of Defense

Title: Acoustic startle eye blink response in veterans with and without anger and aggression related problems

Authors: *L. HEESINK[1,2], R. KLEBER[3], L. VAN BEDAF[1,2], M. HÄFNER[3], E. GEUZE[1,2]; [1]UMC Utrecht, Utrecht, Netherlands; [2]Res. Ctr. Military Mental Hlth. Care, Ministry of Def., Utrecht, Netherlands; [3]Utrecht Univ., Utrecht, Netherlands

Abstract: INTRODUCTION: After deployment, anger is a common mental health problem [1] that is associated with a heightened arousal level. The startle response is an eye blink in response to an (acoustic) startle stimulus and is frequently used in psychophysiology research and several clinical settings to measure arousal [2]. Although it is probable that veterans with anger problems show increased arousal, and thus an increased startle response, this hypothesis remains to be tested. METHODS: 40 male participants with and without anger and aggression problems were recruited for this project. The experiment consists of 20 pulses of white noise of 105 dB, half of them preceded by a less intense prepulse. The pulses were presented randomly to the participants, with a random inter-trial interval length. Startle responses were measured by the use of electromyography (EMG). Two electrodes were placed on the skin surface above the orbicularis oculi muscle [2], using the Biopac MP150 system. Data analysis of the raw EMG signal was performed using AcqKnowledge version 4.3. Both the startle reponse and the habituation effect are analyzed. RESULTS: Preliminary results in 15 veterans with anger and 15 control veterans pointed towards a slightly increased startle response in veterans with anger problems compared to the control veterans. Data collection and analysis is still ongoing. CONCLUSIONS: This study will reveal whether anger and aggression is related to an increased startle response, indicating heightened arousal. This increases our understanding of the neurobiology underlying anger and aggression. *References* [1] Elbogen, E. B., Wagner, H. R., Fuller, S. R., Calhoun, P. S., Kinneer, P. M., & Beckham, J. C. (2010). Correlates of anger and hostility in Iraq and Afghanistan war veterans. *Am J Psychiatry, 167*(9), 1051-1058. [2] Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel, A.

(2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology, 42*(1), 1-15.

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Poster

742. Human Emotion: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NIH grant MH086709

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NIH grant MH084961

Title: Clustering coefficient during resting state predicts trait anxiety in healthy adults

Authors: X. YOU, E. GORDON, M. NORR, M. STOLLSTORFF, *C. J. VAIDYA;
Dept Psychol, Georgetown Univ., Washington, DC

Abstract: Anxiety can be defined as an unpleasant state of mental uneasiness or concern that is accompanied by physical discomfort. An anxious temperament (termed trait anxiety) is a risk factor for mood disorders and has been associated with individual differences in activation of medial prefrontal cortex (PFC) and amygdala, regions involved in emotional processing. Resting-state functional networks involving those regions have been associated with trait anxiety using region-of-interest methods. Whether voxel-wise data-driven methods reveal those same associations is not known. Here, we examined whether a voxel-wise graph theory metric of clustering coefficient is sensitive to individual differences in trait anxiety (measured by the State-Trait Anxiety Inventory (STAI). Clustering coefficient is the probability that the neighbors of the local node are also connected to each other, as a measure of local connectivity or “cliqueness” of a graph. High clustering is associated with robustness of a network, which is resilience against random network damage. We predicted that trait anxiety will correlate with clustering coefficient in medial PFC and amygdala. Eighty undergraduates (ages 18-22) completed the STAI and underwent fMRI at 3T for 5 min in a resting state with eyes closed. 152 fMRI images were slice-time- and motion-corrected, normalized, smoothed with Gaussian kernel with FWHM of 8mm in

SPM8, and bandpass filtered (0.01-0.1Hz). The images were down-sampled to 6 mm voxel size for computational efficiency. Time course of each voxel was correlated to every other voxel and thresholded at p=.001 FDR corrected (r > .32). Clustering coefficient maps were computed on the graph using the brain connectivity toolbox (https://sites.google.com/site/bctnet/measures/list). These maps were entered into a regression with STAI trait anxiety scores entered as the covariate of interest and mean framewise displacement as a covariate of no interest (p < .05 monte carlo corrected). As predicted, higher trait anxiety scores were associated with higher clustering coefficient in ventral and dorsal medial PFC (BA10) extending to anterior cingulate (BA 32), regions implicated in emotional processing and monitoring. Contrary to prediction, a positive correlation was also observed in bilateral parahippocampus/fusiform area and visual cortex (BA 17/18), regions involved in memory and visual processing. Together, more robust information processing - high local connectivity of the graph in these regions during a task-free state may suggest the higher vigilance/monitoring behavior that accompanies higher emotional reactivity in people with higher trait anxiety.

Disclosures: X. You: None. **E. Gordon:** None. **M. Norr:** None. **M. Stollstorff:** None. **C.J. Vaidya:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.07/RR24

Topic: F.01. Human Cognition and Behavior

Support: DFG Grant ZL 59/2-1

Title: Context dependent fear conditioning for de-novo stimuli in spider-fearful individuals

Authors: *A. ZLOMUZICA[1,2], C. MOSIG[2], D. ADOLPH[2], J. MARGRAF[2];
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Abstract: There is considerable evidence for alterations in the acquisition and extinction of conditioned fear in several anxiety disorders, i.e. PTSD, panic disorder and agoraphobia. The underlying mechanisms of altered fear conditioning processes in pathological anxiety however are still a matter of research. Fear conditioning is a complex multi-level process. Studies in healthy humans and non-human animals showed that conditioned fear responses can reoccur

after extinction learning when the excitatory conditioned stimulus is presented in a novel context (fear renewal). Renewal after extinction learning in experimental settings corresponds to one form of relapse after exposure therapy. So far, it is not clear whether fear renewal is also existent in anxious individuals and/or patients with anxiety disorders. In this project, we aimed to fill this gap and therefore assessed alterations in context-dependent fear conditioning in spider fearful individuals by using a virtual reality based approach. We used a differential fear conditioning paradigm during which disorder irrelevant conditioned stimuli were presented within the virtual reality. Our data point towards alterations in fear acquisition and extinction as well a context-dependent renewal of fear in spider-fearful individuals. Our findings have clinical implications for future research aimed at developing new treatment procedures and might contribute to identifying potential ways to minimize relapse after successful exposure treatment.

Disclosures: **A. Zlomuzica:** None. **C. Mosig:** None. **D. Adolph:** None. **J. Margraf:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.08/RR25

Topic: F.03. Motivation and Emotion

Title: The genetics and neurobiology of romantic relationship satisfaction- on the positive side of serotonin transporter gene

Authors: *S. LUO[1,2], D. YU[3], S. HAN[3,2];
[1]Peking University, Dept. of Psychology, Beijing, China; [2]PKU-IDG/McGovern Inst. for Brain Res., Beijing, China; [3]Dept. of Psychology, Peking Univ., Beijing, China

Abstract: Accumulating evidence suggests discrepant negative affect and underlying neural mechanisms in short and long allele carriers of the serotonin transporter promoter polymorphism (5-HTTLPR). The present study investigated whether 5-HTTLPR is associated with positive affect (i.e., romantic relationship satisfaction) and how such associations, if exist, are mediated by the neural activity in brain regions related to emotion and regulation. An fMRI-adapted version of the original Cyberball game was used to assess brain activations during social exclusion in 24 short-short (s/s) homozygotes and 24 long-long (l/l) homozygotes. Participants completed the Relationship Assessment Scale and Interaction Anxiousness Scale after fMRI scanning. l/l compared to s/s group reported significantly higher romantic relationship satisfaction and lower social interaction anxiousness. Social exclusion induced increased

activations in the anterior cingulate (ACC), medial prefrontal cortex, bilateral anterior insula, bilateral superior temporal sulcus, bilateral thalamus and cerebellum in both genotype groups. However, l/l carriers showed stronger activation in the right ventral prefrontal cortex (RVPFC) relative to s/s carriers and the RVPFC activity mediated the association between 5-HTTLPR and romantic relationship satisfaction. Moreover, l/l compared to s/s carriers exhibited increased functional connectivity between the dorsal and rostral ACC, and both the RVPFC activity and dorsal-rostral ACC connectivity mediated the association between 5-HTTLPR and interaction anxiousness. Our findings suggest associations between 5-HTTLPR and both positive and negative aspects of social affect and these associations are mediated by neural mechanisms related to emotion and regulation.

Disclosures: **S. Luo:** None. **D. Yu:** None. **S. Han:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.09/RR26

Topic: F.01. Human Cognition and Behavior

Title: Sex differences of the pleasantness level of pictures upon cerebral activity using fmri

Authors: *A. OMURA, M. TANAKA, U. YAMAMOTO, T. HIROYASU;
Doshisha Univ., Kyotanabe, Kyoto, Japan

Abstract: [Purpose] The goal of this study was to identify an indicator of the level of pleasantness based on information provided by functional brain mapping. The purpose was to verify differences in regions of brain activity and the level of activation owing to sex, because it is considered that work of emotion changes in factors of age and sex. [Methods] In this study, using a visual experiment whose stimulus pictures have the level of pleasantness, we considered sex differences in regions of brain activity and the level of activation based on the levels of pleasantness. Fifteen healthy right-handed subjects (8 women and 7 men) participated in this study. The group had a mean age of 21.2 years old (standard deviation, 0.4 years). We defined the level of pleasantness was categorized into three levels: High, Medium, and Neutral. Stimulus pictures which were sorted by the levels of pleasantness were presented to the subjects, and we measured Blood Oxygenation Level Dependent (BOLD) signal using Magnetic Resonance Imaging (MRI). Obtained data was analyzed by group analysis. Subsequently, we examined the differences in regions of brain activity and the level of activation owing to sex. [Results] The

result on women showed tendency to activate in the cingulate cortex and the frontal lobe rather than that on men. Only in the case of showing pictures with high scores (High), women had activation in the cingulate cortex. On the other hand, in the case of Medium and Neutral, they did not have activation in the cingulate cortex. Besides, as the level of pleasantness decreased, the activation in the frontal lobe became smaller. On the other hand, men did not have activation in the cingulate cortex in the case of High. As with women, a lower level of pleasantness was associated with a smaller activation in the frontal lobe. However, compared with women, they had a tendency for activation in the frontal lobe to be small. The frontal lobe is related to attention and interest. As a higher level of pleasantness induces higher attention and interest, it is considered that a higher level of pleasantness yields a larger active region. The level of pleasantness in sex is considered to reflect in the frontal lobe as the extent of activity, because a previous study has reported that women had more intense emotion than men did. [Conclusions] In this study, we investigated the differences in regions of brain activity and the level of activation owing to sex. In both men and women, a lower level of pleasantness was associated with smaller activated areas in the frontal lobe. Furthermore, men had lower activation than women. Consequently, it is suggested that level of pleasantness and sex differences could be identified.

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Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Support: NICHD 033113

NINDS 22343

Oak Tree Philanthropic Foundation

NSF Graduate Research Fellowship Program 00039202

Title: Characterizing the associations and dissociations between the anatomical structure of the mirror neuron system and social functioning in williams syndrome

Authors: *R. NG[1,2], M. ERHART[3], T. T. BROWN[4,3], A. M. JARVINEN-PASLEY[2], E. HALGREN[5,3], J. R. KORENBERG[6], U. BELLUGI[2];
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Abstract: Williams syndrome (WS) is a disorder determined by a microdeletion on chromosome 7q11.23. Those with WS are best known for their overfriendly demeanor, heightened empathy, and propensity to approach others. In contrast, those with autism spectrum disorder (ASD), a neurodevelopmental condition considered to produce an opposing social profile relative to WS, typically exhibit social withdrawal. Despite robust discrepancies in their social manifestations, both disorders commonly share deficits in social cognition and the ability to develop and maintain peer relationships. Importantly, recent investigations have indicated that those with WS and ASD demonstrate impairment in similar facets of social functioning. Given our understanding of the genetic basis of WS, this condition presents a promising opportunity to explore gene, behavior, and brain relations to better understand neural mechanisms that may undergird social disorders such as ASD. The mirror neuron system (MNS), a neural network implicated in the observation, imitation, decoding, and implementation of actions, has been extensively studied in in ASD. In individuals with ASD, perturbed connectivity and structural anomalies in the MNS are associated with their deficiencies in emotion understanding, theory of mind, empathy, and social communication, and general symptom severity. Considering individuals with WS and ASD paradoxically manifest shared deficits in social functioning (e.g., theory of mind) yet diverge in other respects (e.g., empathy), an investigation to clarify the neural integrity of the MNS in WS is warranted. The present study employed MRI methods to examine the anatomical differences in the MNS substrates in adults with WS (N=20) versus TD controls (N=16). Eighteen participants with WS completed the Social Responsiveness Scale, an inventory typically used to assess social deficits in ASD. Individuals with WS showed reduced cortical surface area of all structures related to MNS and cortical thinning exclusively in pars opercularis relative to TD adults. Associations between cortical thinning in inferior parietal cortex and anterior cingulate cortex with deficits in social communication, social awareness, social cognition, and autistic mannerisms (e.g., repetitive behaviors) were found; however, social motivation (i.e., propensity to initiate interpersonal interactions) was not related to anatomical features of the MNS. Altogether, these finding indicate the social deficits typical to both ASD and WS may be attributed to cortical thinning and diminished surface area in the MNS; whereas, the unusual social drive marked in WS may be distinct from this neural network.

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Poster

742. Human Emotion: Individual Differences and Disorders

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.11/RR28

Topic: F.01. Human Cognition and Behavior

Title: Tolcapone modulates neural circuits underlying emotion processing in patients with schizophrenia and healthy volunteers

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Abstract: Introduction: Functional magnetic resonance imaging (fMRI) studies have identified neural networks involved in emotion processing. However, few studies have explored the effects of dopaminergic drugs on neural circuits underlying emotion regulation in patients with schizophrenia (SCZ). The amygdala is known to be an area involved in the expression of affect, and lesions of this area are known to interfere with normal expressions of mood and social interactions1. Furthermore, perceptual processing of fearful and angry facial expressions is known to be associated with amygdala activation and pharmacological manipulations through drugs known to modulate catecholamines including dopamine alter amygdala reactivity to fearful stimuli2,3. Previous studies indicate that tolcapone, a blood-brain barrier penetrating drug that increases cortical DA levels via COMT enzyme inhibition, modulates a prefrontal cortical neural circuit during working memory4. Here, we explored the effects of tolcapone on neural circuits underlying emotion regulation in a sample of healthy volunteers (HVS) and SCZ. Methods: Thirty-seven subjects (19 HVS and 18 SCZ) were enrolled in this double-blinded, placebo controlled, counter-balanced trial with tolcapone. All subjects with good imaging and performance data were matched on age, gender, IQ, race, handedness, and drug starting condition. Each subject completed the positive and negative symptom scale (PANSS) and the Hamilton anxiety scale (HAM-A) scale on each test day. Subjects then underwent 3T blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) while performing an emotion processing task (Faces Task)3. Results: PANSS data revealed significant

differences between cohorts with SCZ reporting significantly more symptoms than HVS ($p<0.05$). Additionally, in both cohorts, BOLD fMRI revealed that tolcapone significantly modulated neural activity in the left amygdala during the Faces Task by decreasing activation on tolcapone as compared to placebo ($P<0.05$ SVC FWE-correct). Although not statistically significant, the HAM-A scale supports this reduction in amygdala activity, with both groups reporting a trend for decreased anxiety on tolcapone ($p=0.09$). Conclusion: These results suggest that dopaminergic drugs, which increase cortical DA levels via COMT inhibition, can modulate emotional behavior. Further analysis using functional connectivity approaches is underway to further explore whether dopamine is modulating the amygdala directly or through top-down regulation. Further work with larger sample size is also warranted to explore drug X COMT genotype interaction.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Intramural Support.

Title: Variation in the Williams syndrome GTF2I gene and anxiety proneness interactively moderate emotionally aversive event-related prefrontal cortical response

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Abstract: Background: Copy number variation (CNV) in the general transcription factor gene, GTF2i, located in the 7q11.23 region that is hemideleted Williams syndrome (WS) and duplicated in the 7q11.23 duplication syndrome (Dup7), is associated with gene-dose-dependent

anxiety in mouse models and in both WS and Dup7 (Mervis et al. 2012). These results implicate GTF2i in the heritability of human anxiety, but a neurogenetic basis remains unknown. We tested whether sequence variation in this gene, specifically the single nucleotide polymorphism rs2527367 interacts with trait anxiety to collectively impact neural response to anxiety-laden social stimuli. Methods: 260 healthy human adults (115 major allele T/T homozygotes, 118 T/C heterozygotes, and 27 minor allele C/C homozygotes) completed fMRI and the tridimensional personality scale of harm avoidance (HA), a trait measure of anxiety-proneness (Cloninger 1987). During each fMRI event, participants watched a trio of faces and matched a target face to one of two others on the basis of emotional content (anger or fear). Emotion matching events were contrasted with geometric shape matching to map neural substrates of innate social anxiety (Hariri et al., 2003). A first-level analysis was followed by a second-level assessment of neural response to aversive cues and its modulation by GTF2i variation and HA. A factorial model (3-levels of GTF2i) using HA as regressors, and sex, age and IQ as covariates was used to compute how GTF2i and HA interactively impact brain response to aversive cues. Results: We found a main effect of aversive content on amygdala and DLPFC response ($p < 0.05$ FWE corrected). HA scores predicted DLPFC response to aversive content ($p < 0.005$) and GTF2i and HA interactively impacted DLPFC response to aversive cues. By extracting DLPFC values per GTF2i genotype group and using HA scores as regressors, we found a stepwise GTF2i-HA association: T/T variants showed a negative HA-predicted DLPFC response ($t = -2.59$, $p = 0.011$), T/C variants showed no HA effect ($t = 1.25$, $p = 0.21$) while C/C variants showed a positive HA-predicted DLPFC response to aversive cues ($t = 2.91$, $p = 0.0076$). Conclusion: We found an interactive effect of GTF2i variation and trait measures of anxiety-proneness on DLPFC response to aversive social cues in a large healthy adult cohort. In line with gene-dosage-sensitive effects of GTF2i on anxiety (Mervis et al. 2012), our findings illustrate that sequence variation, like CNV, in the GTF2i gene influences the relationship between trait anxiety and brain response to aversive social cues, thereby identifying biomarkers that may guide the search for new pharmacotherapeutic targets for anxiety.

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Poster

742. Human Emotion: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NRF Korea Grant NRF-2006-200512

Title: Sex differences in alpha-frequency EEG responses to preference for facial attractiveness

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Abstract: Electroencephalogram (EEG) responses to faces have been investigated to understand neural mechanisms underlying cortical facial processing. Yet, it is unknown whether there is any sex difference between EEG responses when humans perceive preferred versus non-preferred facial stimuli. The present study examined sex differences in human cortical oscillations, especially alpha (8 ~ 12 Hz) oscillations, during viewing preferred versus non-preferred faces. Sixteen healthy volunteers performed a series of tasks, including passive viewing and active choice tasks, with a set of 24 face photographs. During the passive viewing task, participants passively watched a presented face stimulus for 1 s. In the second active choice task, participants saw two consecutive face stimuli and selected a more attractive face. The preference level of each face stimulus was evaluated by the number of times being selected. The preferred and non-preferred face stimulus groups were determined based on the preference levels. 64-channel EEG signals were recorded during both tasks. We analyzed the alpha power and phase synchronization from EEG signals recorded in the passive viewing task (no explicit preference evaluation was made). A complex wavelet transform and a phase-locking value (PLV) were used to estimate the alpha power and phase synchrony, respectively. The differences of power and phase synchrony between preferred and non-preferred groups were statistically assessed by the repeated ANOVA and t-test with respect to the effect of sex, cortical regions and peri-stimulus short-time periods. The results revealed that male and female participants exhibited different spatio-temporal patterns of differences between preferred and non-preferred face stimuli. In male participants, differences of alpha power between preferred and non-preferred face stimuli were dominantly found in the occipito-parietal areas during the early (0.15 ~ 0.35 s after stimulus onset) and mid periods (0.35 ~ 0.55 s). By contrast, in female participants, differences were found in the frontal and centroparietal areas during the mid and late (0.55 ~ 0.75 s) periods. Similarly, large-scale phase synchrony across the frontal and centroparietal areas appeared in the mid and late periods in female participants whereas there was no clear large-scale phase synchrony in male participants. Our results suggest that females might involve more high-level processes including emotional networks in the evaluation of preferred versus non-preferred faces compared to males. Our results may also raise a possibility to predict favorable faces only using human EEG signals.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIMHD G12 MD007599-28

NIDA R24 DA012136-12

Title: Sympathetic reactivity and trauma-related symptoms differentially predict hypervigilant neural responses to novelty

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Abstract: Elevated basal arousal is one of the key symptoms of post-traumatic stress disorder (PTSD), and affective brain areas (i.e., limbic and prefrontal cognitive control regions) are hyper-reactive in people with PTSD (e.g., Bryant et al., 2005). In addition, trauma-exposed people often experience hypervigilance for threat in the environment, which can be reflected in a heightened affective brain response to ambiguous (i.e., neutral), and novel information. Normatively, affective brain regions activate to novelty independently of valence and arousal (e.g., Weierich et al., 2010). The amygdala response to novelty is heightened in higher arousal states (e.g., Schwartz et al., 2003), suggesting that the neural response to novelty might be a strong index of hypervigilance. In addition, sympathetic nervous system reactivity indexed by salivary alpha amylase (sAA) is heightened in people with trauma exposure (e.g., Ali & Pruessner, 2012). We tested the hypothesis that sympathetic reactivity would predict hypervigilance as indexed by amygdala and cognitive control region (rostral middle frontal gyrus; rostral anterior cingulate cortex; orbitofrontal cortex) activation to novel information in trauma-exposed adults. Seventeen women with a history of trauma exposure provided saliva samples before and during a structured clinical interview. The interview included discussion of trauma exposure and served as a trauma reminder. We calculated sAA reactivity as the difference between pre- and mid-interview levels. In a later session, participants completed an fMRI scan, during which they viewed novel and familiar affective scenes. We used *a priori* anatomically-defined regions of interest to test the utility of sympathetic reactivity and PTSD symptoms in predicting hypervigilance as indexed by greater limbic and control region activity

in a Novelty (novel, familiar) by Valence (positive, negative, neutral) factorial design. SAA reactivity predicted right amygdala activation for novel neutral scenes (r = .525, p = .031), suggesting that heightened sympathetic reactivity to trauma reminders predicts hypervigilance as indexed by the limbic response to ambiguous, uncertain information. In addition, self-reported PTSD symptoms predicted right rostral middle frontal gyrus activation to novel neutral (r = .585, p = .014) and novel negative scenes (r = .572, p = .016), suggesting an over-recruitment of the cognitive control system in people with trauma exposure. Together, these data suggest that sympathetic reactivity during trauma reminders might be a marker for hypervigilance to potentially threatening information in trauma-exposed people.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: ONR Grant N0014-04-1-005

Title: Euphoric experience is related to variability in left medial orbitofrontal cortex volume

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Abstract: Research suggests that medial portions of the human orbitofrontal cortex are associated with reward processing and positive affect, while the lateral orbitofrontal cortex is linked to negative affect. Recent research has shown that variability in the volume of the medial, but not lateral, orbitofrontal cortex is associated with variability in the expression of positive, but not negative, affect. However, little is known about the subjective experience of extreme joy or euphoria and how this state may relate to the medial orbitofrontal cortex. One particularly captivating hypothesis is that variability in euphoric experience is related to variability in the structure of the medial orbitofrontal cortex. To test this hypothesis we measured participants' self-report feelings of euphoria in response to a highly anticipated first time skydive and measured orbitofrontal cortical volumes with structural magnetic resonance imaging. We recruited 31 individuals who independently contacted a local skydiving school to schedule their first tandem skydive. Testing took place over two time-matched days: (1) skydive day and (2)

control day. Self-report euphoria and anxiety levels were collected on the plane immediately prior to jumping and at the airfield immediately after landing. Baseline levels of euphoria were collected on the control day at matched time-points. Participants underwent T1-weighted structural magnetic resonance imaging in a 3-Tesla MRI on the control day. We performed cortical reconstruction and volumetric segmentation with the Freesurfer image analysis suite. We tested for a positive association between euphoric experience and OFC volume across left-right and medial-lateral dimensions (adjusted $\alpha = 0.0125$; i.e., $P = 0.05/4$ comparisons). We controlled for the effects of age, gender, state anxiety, and intracranial volume. Compared to baseline levels, skydiving elicited a large increase in self-reported euphoria ($F1,30 = 32.04$, $P = 0.000004$). Variability in participants' left medial orbitofrontal cortex predicted euphoria both prior to jumping ($r = 0.44$, $P = 0.01$) and at landing ($r = 0.46$, $P = 0.008$). Such effects were not observed in other OFC regions (P-values > 0.1). Thus, euphoric experience is associated with the structure of the left medial orbitofrontal cortex such that, the greater the volume, the greater the euphoria.

Disclosures: J.M. Carlson: None.

Poster

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James J Peters Veterans Affairs Medical Center

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Title: The neural mechanisms of anticipation and implementation of cognitive reappraisal in avoidant personality disorder patients

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York, NY; [4]Inst. of Psychology, Chinese Acad. of Sci., Beijing, China; [5]Psychology, Columbia Univ., New York, NY; [6]James J Peters VA Med. Ctr., Bronx, NY

Abstract: Avoidant personality disorder is characterized by pervasive anxiety and fear of rejection in anticipation of and during exposure to social situations. An important but underexplored question concerns whether anxiety in avoidant patients is associated with an impaired ability to engage emotion regulatory strategies before and during appraisal of negative social stimuli. We aimed to address this question by examining the neural mechanisms underlying the use an adaptive emotion regulation strategy, cognitive reappraisal, in this population. The activity of regions previously associated with negative emotion reactivity, including the amygdala, was of particular interest. We assessed state and trait anxiety levels and measured neural activity via functional magnetic resonance imaging both in anticipation of and during performance of an image-based reappraisal task in 17 avoidant patients and 21 healthy participants. As expected, avoidant patients showed greater state and trait-related anxiety relative to healthy participants. In addition, relative to healthy participants, avoidant patients showed pronounced amygdala hyper-reactivity during reappraisal anticipation as well as hyper-reactivity in right amygdala during negative image appraisal itself. Further, in avoidant patients these hyper-reactivity effects were positively associated with increasing self-reported anxiety levels. No group differences in reappraisal-related activity were identified. These results suggest that amygdala reactivity may represent a key component of the neural mechanisms underlying the heightened anxiety present in avoidant patients.

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Poster

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Oak Tree Foundation

Title: Music and emotion in adults with williams syndrome: Expressivity toward affective musical pieces through nonverbal channels of behaviors

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Abstract: Williams Syndrome (WS) is a rare neurodevelopmental genetic disorder, characterized by a hemizygous deletion of about 25 adjacent genes on the long arm of chromosome 7. Of interest to cognitive scientists is the uneven cognitive profile that is characteristic with WS. In spite of an average full scale IQs ranging around 55 to 60, a complex pattern of weaknesses and strengths is found in other cognitive abilities (Bellugi et al., 2000). Weaknesses include visual-spatial cognition and conceptual reasoning abilities; on the other hand, cognitive strengths are found in their linguistic abilities and facial processing. Musical affinity in WS has been suggested to be a particular strength in their profile; in a parental questionnaire study, it was noted that WS individuals display greater emotional responses to music and manifest interest in music at an earlier age (Levitin et al, 2005). To better understand the musical profile of individuals with WS, the present study examined behavioral responses to novel musical pieces. In the study, adult participants (WS=22; TD=22) listened to ten emotion-eliciting musical pieces (happy, sad, or scared) for 20 seconds each. Results for this task revealed that on average, the WS group produced longer duration of eye contact (EC) to the experimenter (24 sec. vs. 3 sec.) as well as more rhythmic movements to the music (RTM) compared with the TD population (28 sec. vs. 5 sec.); furthermore, these nonverbal modalities of expressivity in the WS group were especially heightened during the happy pieces compared with the music of other emotions. In addition, co-occurrences of nonverbal behaviors were investigated to observe whether multiple channels of expressivity would manifest during this task and whether specific affective musical pieces would drive this effect. Co-occurrences of EC and facial expressions (FE) were statistically significant for the happy and sad musical pieces with more expressivity expressed in the WS group than the TD group. For co-occurrences with RTM and EC, a significant difference was observed for only the happy musical pieces, as the WS group was once again more expressive than the TD group. On the whole, the WS group was more expressive through nonverbal channels of RTM and EC compared to the TD group when listening to different types of musical pieces ($p < 0.05$). This study provides evidence that expressivity observed in children with WS in social tasks extends into adults in this nonverbal, auditory task. This study serves to further characterize this distinct social profile of individuals with WS, probing responsivity to music as a unique characteristic of the WS phenotype.

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Poster

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Support: NIH Grant R21MH098198-01

Title: Relationship between subacute post-concussion symptoms and posttraumatic stress symptoms of survivors with minor physical injury

Authors: *B. DUNCAN[1], H. XIE[2], E. F. M. OLDS[3], K. R. BRICKMAN[4], M. B. TAMBURRINO[3], K. BRENNER[5], S. WATSON[5], B. P. KAMINSKI[5], M. MATTIN[5], E. FERGUSON[5], X. WANG[3];
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Abstract: Mild traumatic brain injury (mTBI) occurs frequently as a result of motor vehicle collision (MVC). Survivors with mTBI experience significantly more symptoms in the subacute stage (72 hours to 3 months) post-MVC. Increasing studies reveal the association between physical conditions and posttraumatic stress disorder (PTSD) symptoms after mTBI, but few of these studies focus on survivors with minor physical injuries. We explored the relationship between the progression of post-concussion symptoms and of PTSD symptoms in survivors with minor physical injury over the initial three months after MVC. A prospective study recruited MVC survivors with minor physical injuries (An Abbreviated Injury Scale < 3). mTBI was diagnosed according to The American Congress of Rehabilitation Medicine criteria. Patients were evaluated with the Rivermead Post-Concussion Symptoms Questionnaire for the post concussion symptoms (PCS) and PTSD Checklist (PCL) for traumatic stress symptoms at 2 weeks, 1 month and 3 months after MVC. The univariate ANOVA was used to compare PCL scores between mTBI and non-mTBI group. Partial correlation analysis was used to examine the relationship between PCL and PCS scores, controlling for age and gender. 52 mTBI and 78 non-mTBI patients were recruited. In the mTBI group, the results reported higher PCL subscores of hyperarousal than non-mTBI group (F=5.832, P=0.024, N=14:13) at 3 months after MVC, but not significant difference in total PCL score. Within mTBI survivors, the PCS scores were positively correlated with the PCL total scores at initial days (r=0.407, P=0.026, N=34), 1 month (r=0.556, P=0.026, N=19) and 3 months (r=0.806, P=0.002, N=14) after MVC. Further analyses

revealed PCS scores were positively correlated with PCL subscores of hyperarousal at 1 month (r=0.615, P=0.011, N=19) and 3 months (r=0.817, P=0.001, N=14); and PCL subscores of re-experiencing (r=0.694, P=0.012, N=14) and avoidance (r=0.676, P=0.016, N=14) at 3 months after MVC. mTBI did not significantly elevate PTSD symptoms of MVC survivors with minor physical injury in the initial three months after MVC. However, post-concussion symptoms associated with hyperarousal, re-experiencing and avoidance persisted months after MVC. The current cohort of MVC survivors with mTBI demonstrates a similar association between PCS and PTSD symptoms as other mTBI populations.

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Poster

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Support: NIH Grant R21MH098198-01

Title: Mild traumatic brain injury affects cortical activation and substrates of negative emotion processing

Authors: *H. XIE[1], X. WANG[2], A. COTTON[2], M. B. TAMBURRINO[2], K. R. BRICKMAN[3], K. BRENNER[4], N. SELLERS[4], U. BHAVSAR[4], E. FERGUSON[4], S. HO[5], I. LIBERZON[5]; [1]Neurosciences, [2]Psychiatry, [3]Emergency Med., Univ. of Toledo, Toledo, OH; [4]ProMedica Hlth. Syst., Toledo, OH; [5]Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: Mild traumatic brain injury (mTBI) patients frequently suffer from symptoms of post traumatic stress disorder (PTSD) and depression. Functional magnetic resonance imaging (fMRI) studies reveal that deficits in brain activation that are associated with negative emotion processing contribute to these symptoms. However, the effect of mTBI on the brain activation that is associated with negative emotion processing has not been addressed. In this study, we compared cortical activation to negative emotional stimulation in motor vehicle collision (MVC) survivors with or without mTBI 7.2±3.1 days after MVC. In addition, cortical thickness in regions where activations differed between two groups was concurrently examined to assess

mTBI effects on cortical substrates of negative emotion processing. MVC survivors were separated into mTBI or non-mTBI groups based on the presence or absence of concussive symptoms documented in Emergency Departments (EDs). A task that involved identifying fearful vs. neutral faces during fMRI was used to detect the cortical activation associated with negative emotion. Moreover, cortical thickness was measured from MRI images using Freesurfer. Compared to the non-mTBI group (n=23), the mTBI group (n=21) had less negative emotion activation in left medial (-7.5, 39.1, -15.2; $p=10^{-3.2}$) and lateral (-14.9,39.1,-19.8; $p=10^{-4}$) orbitofrontal cortex (OFC), and in a left superior parietal gyrus (SPG; -5.9,-81.8,33.8; $p=10^{-3.6}$) at at this early time after MVC. The SPG region was also thinner in the mTBI group (2.20±0.23 mm) compared to non-mTBI group (2.33±0.25 mm; F=12.8, p=0.01), but there was no cortical thickness difference in OFC regions. The results suggest cortical activation that was associated with negative emotion processing was altered in mTBI survivors within days after mTBI. The co-localization of thinning cortical thickness in SPG in mTBIs survivors is consistent with the hypothesis that mTBI alters some cortical substrates for negative emotion processing. Future studies may explore the relationship between effects of mTBI on brain functions and structures and symptoms of PTSD and depression after head injuries.

Disclosures: H. Xie: None. **X. Wang:** None. **A. Cotton:** None. **M.B. Tamburrino:** None. **K.R. Brickman:** None. **K. Brenner:** None. **N. Sellers:** None. **U. Bhavsar:** None. **E. Ferguson:** None. **S. Ho:** None. **I. Liberzon:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.20/RR37

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH094633

Title: Default and salience network intrinsic connectivity in behaviorally inhibited children at risk for anxiety

Authors: *B. C. THOMAS, S. MORALES, K. E. PEREZ-EDGAR;
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Abstract: Behaviorally inhibited (BI) temperament in childhood is associated with perturbed social information processing, such as biased attention to negative stimuli (Perez-Edgar et al.,

2010) and avoidance of negative emotion (Pine, 1999), as well as increased risk for social anxiety (Hirshfeld et al., 1992). One mechanism that may underlie perturbed information processing in children at risk for anxiety is altered intrinsic functional connectivity (iFC; Buckner et al., 2013). Anxious adults exhibit increased iFC in the default mode (PCC-mPFC) and salience (insula-ACC) networks, but iFC in well-established intrinsic networks has not been examined in children at risk for anxiety. Here we collected resting state fMRI data from BI (n=13) and non-BI (n=20) children to study the relationship between BI and iFC in mid-childhood (ages 9-12). Data were collected on a Siemens Magnetom Trio 3T scanner (6 minute run; 180 volumes; TR=2s; TE=30ms; flip=70; FOV=220x135mm; voxel=2.3x2.3x5). Preprocessing was performed in SPM8 and the CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). Whole-brain seed based functional connectivity analyses were then performed in the CONN toolbox with resulting maps of Fisher-Z transformed correlation coefficients cluster-level FDR-corrected to $p<0.05$. Salience network seeds were placed in left and right insula, and a DMN seed placed in mPFC. For the BI versus non-BI group (i) left and right insula connectivity was greater to vmPFC but less to the typical salience node of dorsal ACC and (ii) mPFC connectivity was greater to PCC and right dlPFC. Results show that BI children exhibit a shift in salience network iFC from dorsal to ventral medial PFC, and greater iFC of mPFC with default and executive regions. This suggests that a bias in salience network iFC toward self-referential and affective processing (vmPFC) may be an underlying neural mechanism for perturbations to social information processing and risk for anxiety in BI. Moreover, results suggest that mPFC, a central node in self-referential and affective networks, may play an increased role in coordinating intrinsic neural functioning in BI.

Disclosures: B.C. Thomas: None. **S. Morales:** None. **K.E. Perez-Edgar:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: MH094633

Title: EEG asymmetry moderates the relation between behavioral inhibition and social anxiety

Authors: *S. MORALES[1,2], B. C. TABER-THOMAS[2], N. THAI[2], K. E. PÉREZ-EDGAR[2]; [1]Penn State Univ., State College, PA; [2]Pennsylvania State Univ., University Park, PA

Abstract: Behavioral inhibition (BI) is a temperament type characterized by displays of fear in response to novelty, such as heightened vigilance and avoidance of unfamiliar adults, objects, and situations. BI is one of the strongest predictors of anxiety, particularly social anxiety disorder (Claus & Blackford, 2012; Pérez-Edgar & Fox 2005). However, not all BI children develop anxiety disorders; this suggests that additional internal or external factors moderate the relation between BI and anxiety. Identifying these factors will help to successfully identify which fearful children are at greatest risk. Asymmetrical electroencephalogram (EEG) activity in the frontal cortex has been proposed as one of such factors (Degnan & Fox, 2007). For example, BI remains stable across childhood, if coupled with greater right frontal asymmetry (Fox et al., 2001). Based on these findings, the current study evaluates whether the relation between BI and social anxiety symptoms is moderated by frontal EEG asymmetry in middle-childhood. EEG data at rest were provided by 43 children as part of an ongoing study (Mage=10.3; 22 BI; 19 boys). Asymmetry scores are derived by subtracting activity at frontal electrodes on the right hemisphere (F4) from a corresponding site on the left hemisphere (F3) (negative = more right asymmetry; M=-0.07, SD=0.14). Children were characterized as BI based on maternal reports on the Behavioral Inhibition Questionnaire (Bishop et al., 2003). Mothers also reported on the Social Anxiety Scale for Adolescents (SAS-A) to assess anxiety (La Greca & Lopez, 1998). The SAS-A is composed of the following sub-scales: fear of negative evaluation (FNE); social avoidance and distress in new situations (SAD-New); and generalized social avoidance and distress (SAD-General). We found that the expected negative relation between EEG asymmetry and social anxiety symptoms differed by BI status (interaction p = .018) - such that greater relative right EEG activity was related to more anxiety symptoms only for BI children (r=-.45 vs. r=.29). Moreover, when doing the same analyzes for each of the SAS-A sub-scales, results were only significant for the SAD-New sub-scale (interaction p = .031). These results are in line with previous literature, showing that only BI children with greater right-sided asymmetry display high levels of anxiety (Fox, Calkins, & Bell, 1994). In addition, the results highlight that this well-studied relation between EEG asymmetry, BI, and anxiety may be driven specifically by temperament-linked concern with novelty, rather than general facets of anxiety.

Disclosures: S. Morales: None. **B.C. Taber-Thomas:** None. **N. Thai:** None. **K.E. Pérez-Edgar:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH094633

Title: Neural processing of social stimuli is associated with affective decision making on the Iowa Gambling Task

Authors: *N. THAI, B. C. TABER-THOMAS, K. E. PÉREZ-EDGAR;
Dept. of Psychology, The Pennsylvania State Univ., University Park, PA

Abstract: Middle childhood reflects a period in development when children begin to make decisions about meaningful life events that carry risk regarding future consequences. For example, children may decide whether or not to socially interact with peers, or engage in behavior that risks social rejection. We also know that the neural processing of social information such as faces is crucial in shaping subsequent social interactions (Leopold & Rhodes, 2010). Existing studies of typically developing participants (Herzmann et al., 2010; Hileman et al., 2011) and participants with autism (Clark et al., 2008) suggest that face processing is related to social cognition skills and real-world social behavior. However, very little is known regarding the underlying mechanism linking altered neural processing of social stimuli to real-world social functioning. In the present study, we explore the notion that social information processing may be associated with affective decision making, a process critical for social functioning, including short- and long-term decision making. Twenty-eight children between the ages of 9 and 12 performed the dot-probe task, a social information (face) processing task in which they were asked to respond to a probe that replaces one of two faces. Face pairs were either Angry-Neutral (A-N) or Neutral-Neutral (N-N) face expressions. The P2 ERP component was measured to capture neural processing related to attending to affective social stimuli (Carretie et al., 2001; Eldar & Bar-Haim, 2010). Children also performed a neuropsychological assessment of affective decision making via the Iowa Gambling Task (IGT; Bechara et al., 1994), which is designed to mimic the complex decisions individuals make in the real world. Preference for long-term advantageous versus disadvantageous options on the IGT is quantified as the long-term consequences index. The P2 amplitude was negatively correlated with long-term consequences indices for both A-N ($r = -.412$, $p = .03$) and N-N face pairs ($r = -.447$, $p = .03$). Results indicate that more evaluative neural processing of faces is associated with poorer affective decision making in children. This may present a mechanism through which altered processing of social stimuli impacts real-world functioning, namely by disrupting affective decision making processes. In this way, the affective decision-making process may be an important target for future prevention and intervention efforts for difficulties with social functioning.

Disclosures: **N. Thai:** None. **B.C. Taber-Thomas:** None. **K.E. Pérez-Edgar:** None.

Poster

742. Human Emotion: Individual Differences and Disorders

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Program#/Poster#: 742.23/RR40

Topic: F.01. Human Cognition and Behavior

Support: University of Colorado Denver's Department of Psychology's Undergraduate Research Fund

University of Colorado Denver's Undergraduate Research Opportunity

Title: Magnitude of visually evoked responses differ across level of altruism

Authors: *J. J. FOWLER[1], K. EVERHART[2], C. PHIEL[3], P. KAPLAN[1], D. S. ALBECK[1]; [2]Dept. of Psychology, [3]Dept. of Integrative Biol., [1]Univ. of Colorado Denver, Denver, CO

Abstract: Emotionally charged images evoke greater neural responses in the brain than neutral images, as measured by scalp electroencephalography. Negatively valenced images evoke the strongest response, followed by positive and then neutral input. Whether or not a person's behavioral traits, in particular altruism, vary with how the brain interprets visual input has not been extensively investigated. In this study we have used event-related potential (ERP) methodology to study the hypothesis that altruism affects ERP. Reaction to standardized images was recorded at three electrode placements known to exhibit strong response to visual input, Fz, Pz, and Cz. Preliminary results indicate that participants with higher levels of altruism had a significantly larger ERP response to negative images recorded at the Cz site ($t(6) = -2.45$, $p < .05$), and the response at the Pz site showed a trend toward a significant difference ($t(6) = -2.25$, $p = .065$) compared to those with low levels of altruism. Recordings at the Fz placement found no differences between groups. No differences across altruism levels were found with presentation of either positive or neutral images. Oxytocin receptor genotype (OXTR) in these participants is also currently being studied.

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Poster

742. Human Emotion: Individual Differences and Disorders

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Program#/Poster#: 742.24/RR41

Topic: F.01. Human Cognition and Behavior

Support: NIMH/FIC R21MH095656 to MAG

Title: Serotonin transporter genotype modulates the effects of dopamine transporter genotype on learning from positive and negative feedback

Authors: *H. KHDOUR[1], J. Y. NATSHEH[2,3], I. T. MUGHRABI[2,4], H. DARWISH[2,5], M. M. HERZALLAH[2,3], M. A. GLUCK[3];
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Abstract: In this study, we investigate the impact of interaction between dopamine and serotonin levels on feedback-based learning. A major modulator of dopamine levels in the brain is the dopamine transporter (DAT) that is coded by the DAT1 gene, while serotonin levels in the brain are modulated by the serotonin transporter (SERT) that is coded by the SLC6A4 gene. We examined the variable number of tandem repeats polymorphism (VNTR) in the 3' untranslated region (3'-UTR) of DAT1 the serotonin transporter polyadenylation polymorphism (STPP) of SLC6A4 in 120 racially homogenous healthy volunteers and grouped them according to DAT1 VNTR genotype into 9-repeat carriers (high dopamine) and 10-repeat homozygotes (low dopamine), and SLC6A4 STPP genotype into short allele carriers (high serotonin) and long allele homozygotes (low serotonin), using a probabilistic category-learning task that allowed for dissociation between the acquisition of positive feedback (reward) and negative feedback (punishment). Our results suggest that genes that modulate dopamine and serotonin levels affected reward learning but not punishment learning. When we held SLC6A4 constant and varied DAT1 genotypes, there was better learning from both reward and punishment with higher dopamine levels (9-repeat carriers) in the context of higher serotonin levels (short allele carriers). Conversely, there was no difference between DAT1 genotypes in learning from positive and negative feedback in the context of low serotonin levels (long allele homozygotes). When we held DAT1 genotypes constant, there were no difference between SLC6A4 genotypes in the context of high (9-repeat carriers) or low (10-repeat homozygotes) dopamine levels. These findings argue in favor of a modulatory role of serotonin on dopamine function. Future studies will investigate this gene-gene interaction in Parkinson's disease and Major Depressive Disorder as it relates to cognitive function and response to treatment.

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Poster

742. Human Emotion: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: Farris Family Foundation Award

Whitehall Foundation Grant

Title: Complex effects of serotonin transporter and BDNF polymorphisms on emotion responsiveness

Authors: ***T. L. GILMAN**[1], M. S. LATSKO[1], L. M. MATT[1], J. J. FLYNN[1], O. DE LA CRUZ CABRERA[2], D. DOUGLAS[1], A. M. JASNOW[1], K. G. COIFMAN[1];
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Abstract: Many studies have observed significant correlations between gene polymorphisms and emotional responsiveness. Far fewer, however, have examined how interactions of polymorphisms relate to adaptive/maladaptive changes in elicited emotional experiences and expressions. Two of the most heavily studied polymorphisms relating to emotion are the serotonin transporter linked polymorphic region (5-HTTLPR) with its associated single nucleotide polymorphism (rs25531), and the brain derived neurotrophic factor (BDNF) Val66Met polymorphism (rs6265). Together, these polymorphisms affect genes integral in neurodevelopment, learning, memory, attention, and emotion. Here, we took an inclusive approach in evaluating the effects of these polymorphisms both individually and together on emotion responsiveness across evocative contexts. Measures included facial behaviors, affect, and autonomic activity. Individuals homozygous for the short allele (S'S') of the 5-HTTLPR exhibited elevated positive emotion during simulated peer rejection (Cyberball) relative to those with at least one long allele (L carriers) present. Significantly elevated positive emotions were also reported in a separate cohort of S'S' subjects observing an emotionally troubling film compared to their L carrier counterparts. This contrasts with BDNF Met carriers whom display persistent negative emotion following both rejection and simulated social acceptance

(Cyberball). Further, presence of the BDNF Met allele modulates several self-reported and facially coded emotions in a 5-HTTLPR-dependent manner in two separate cohorts of subjects. Thus, our data provide evidence that behaviors correlated with polymorphisms can have counterintuitive outcomes when more than one polymorphism is evaluated. Such inclusive approaches as these will enhance knowledge and appreciation of the complexity by which different polymorphisms reciprocally modulate each other to globally influence emotion responsiveness.

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Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.26/RR43

Topic: F.01. Human Cognition and Behavior

Support: Brain Korea 21 Plus Project in 2014

Title: Gender differences in EEG gamma band activity correlated with behavioral responses to different emotional contents

Authors: ***J. YEON**, S.-P. KIM;
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Abstract: Affective computing is a human-computer interaction technology that enables a computer to respond to or influence human emotions. Although it employs various means to recognize affective states of a user, few studies have explored sources in the contents of stimuli that evoke emotions. However, comprehending aroused emotions with its semantic sources would be helpful to develop affective computing technology. Thus, we studied human emotions aroused by various emotional semantic sources. Especially, we hypothesized that emotional responses to each source would be different across the gender. We addressed this hypothesis by analyzing both neurophysiologic and behavioral responses. Four different positive emotional contents, including baby, sports, food, and scenery, were extracted from the analysis of the international affective picture system (IAPS), representing different semantic sources to evoke emotions. Emotional pictures for each content as well as neutral pictures were collected from the in-house affective picture system (a Korean version of IAPS). 20 university students (10 males,

mean age = 23.35 years) participated in the experiment. The 14-channel wireless headset (Emotiv, Inc., Australia) was used to measure electroencephalograms (EEG). In each trial, each participant was shown an emotional or a neutral picture in a random order for 2.5 seconds, followed by self-rating of valence and arousal scores. Short-time Fourier transform was used to estimate time-varying spectral power within 5 frequency bands: Delta (1~3 Hz), Theta (4~8 Hz), Alpha (9~12 Hz), Beta (13~20 Hz), and Gamma (20~50 Hz). Similarity of power changes in each frequency band among all participants was measured using multi-dimensional scaling (MDS). The analysis of the self-rating data revealed that baby and scenery pictures marked lower arousal levels than neutral ones while food and scenery pictures marked higher levels, in both gender groups. The largest gender difference was found in baby pictures, followed by scenery, sports and food in a descending order. Neutral pictures showed the smallest difference as expected. Among the 2D feature maps for each frequency band created by MDS, the gamma band activity (GBA) map exhibited a pattern that best matched self-rating data. In this map, the separability between gender groups was the largest for baby pictures followed by sports, food, scenery and neutral ones. Hence, the order of gender difference in self-ratings and GBA was identical except for scenery pictures. The result suggests that GBA may underlie gender difference in response to various semantic sources of emotional pictures.

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

Poster

742. Human Emotion: Individual Differences and Disorders

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 742.27/RR44

Topic: F.03. Motivation and Emotion

Title: A State of Art on music in aphasia treatment

Authors: *L. JIMENEZ-DABDOUB, C. M. MORÁN-MARTÍNEZ, M. RODRÍGUEZ-ORTIZ;
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Abstract: Language and music are cognitive skills that distinguish human beings from other species. Language can be affected due to different disorders like aphasia. Aphasia commonly results after a traumatic brain injury (TBI). New research in neuropsychological treatments has showed music efficacy for recovery of certain linguistic skills in aphasic patients (Schlaug et al., 2008). In the present work a holistic view is provided from the interaction of Neurosciences and

neuropsychological rehabilitation. A state-of-art looks into the level of knowledge and development reached within a science or a technique according to Harrison Supplee (1910); hence why in order to explore the use of music to treat aphasia and the way how this field of research and application has evolved, a state-of-art was done. 41 articles were selected based on clear criteria; after this procedure it was concluded that the preferred intervention method was Melodic Intonation Therapy (MIT). Publications selected were mostly from USA (41%), then publications coming from Europe (29%), then Canada (14%) and finally, the sum of three Asian countries (14%). The year criteria was 1973-2013, the year 2012 was the one were more articles were found. Articles then were classified by type of study, musical intervention, design, achieved changes in patient's general skills and physiological results. Curiously, most studies were concerned on the same preoccupation the present study has, to get to know the state-of-art of music being used in aphasic patients, this is why, there were only 15 studies found where a literature review was done. Still there were case studies, series of case studies and experimental studies, adding all of this we got a 51% of studies in which MIT was used. There were neurophysiological, neuropsychological, inferential statistics and evidence-based measures taken into account. In spite of believing the growth on this area of interest being related to technological neuroimaging advances, the preference on result presentation was neuropsychological data (29%). It was concluded that aphasics get improvement in words used in phrases, immediate repetition, better rhythm, verbal recognition, prepositional speech, memorization of new words and get motivation to communicate. The importance of truly interdisciplinary work is pointed out where the perspective is taken beyond that of only on the neurological rehabilitation of the patient. Further more, taken into an inclusive level where not only the patient but also their entire social environment' is involved to get the best tools for their reintegration and self-acceptance.

Disclosures: L. Jimenez-Dabdoub: None. **C.M. Morán-Martínez:** None. **M. Rodríguez-Ortiz:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 743.01/RR45

Topic: F.01. Human Cognition and Behavior

Support: BNIG11nov021

Title: Pre-existing brain states predict risky choices

Authors: *Y.-F. HUANG[1], C. SOON[1], O. A. MULLETTE-GILLMAN[2], P.-J. HSIEH[1]; [1]Duke-NUS Grad. Med. Sch., Singapore, Singapore; [2]Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Rational decision-making models assume that people resolve an economic problem based on its properties and the underlying utility. Here we challenge this view by examining whether pre-stimulus endogenous neuronal fluctuations can bias economic decisions. We recorded subjects' pre-stimulus neural activity patterns with fMRI before they chose between pairs of certain outcomes and risky gambles. Our results indicate that activities in the brain regions associated with uncertainty and reward processing can bias subsequent risky decision making, showing that these neuronal activities are involved in biasing subsequent choice selection. This finding challenges theories which propose that choices merely reveal stable underlying distributions of hedonic utility. Endogenous brain states of this sort might originate from a systematic cause or a stochastic type of neural noise, which can be construed as contextual factors that shapes people's decision making.

Disclosures: Y. Huang: None. **C. Soon:** None. **O.A. Mullette-Gillman:** None. **P. Hsieh:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 743.02/RR46

Topic: F.01. Human Cognition and Behavior

Title: Systematic cognitive biases during probabilistic inference

Authors: *J. LIU[1], C. Z. GUO[1], M. A. SALHOLZ-HILLEL[1], A. SOLTANI[2]; [2]Psychological and Brain Sci., [1]Dartmouth Col., Hanover, NH

Abstract: Most naturalistic decision making entails simultaneous learning and integration of information from various sources while feedback is usually in a binary format (e.g. success or failure), requiring the brain to solve the nontrivial problem of assigning the correct weight to each piece of information. Recently, it has been shown that even non-human primates are capable of solving such problems (Yang and Shadlen 2007) and necessary computations can be

done at the synaptic level (Soltani and Wang 2010). However, it is unclear how much of the knowledge acquired through feedback, and reflected in the choice behavior, is consciously accessible to the subject and/or prone to cognitive biases. We tested human subjects in a probabilistic decision making task where on each trial subjects were presented with 4 cues (simple geometric shapes) and asked to make a decision between two alternative options (red and blue targets) in order to get point rewards. Each cue carries a unique weight of evidence (WOE, log likelihood ratio that the cue is presented given either red or blue target is assigned with reward) and the combined WOE from all cues presented on a given trial determines reward probabilities on the two targets. After completing a few blocks of decision trials the subjects estimate how much each cue or a combination of cues predicts reward on one of the two targets (posterior probabilities). We examined how subjects' estimates of posteriors correlated with the actual posteriors and were biased when the probability of reward on the two targets (prior probability) were not equal. We found that while subjects were able to closely estimate posteriors for individual cues or combination of cues, they also showed systematic but contradictory biases when priors were unequal. Specifically, estimated posteriors for individual cues were biased toward the less rewarding outcome but posteriors for combination of cues were biased toward the more rewarding outcome, similarly to the choice behavior. We show that these patterns of results can be explained by assuming that for each cue subjects learn a quantity proportional to the log posterior odds which is also influenced by the log prior odds, and that information from individual cues are combined to make inferences or decisions when more than one cue are presented. In summary, we show that whereas human subjects have conscious access to what they learn through reward feedback, they exhibit systematic biases that reflect what is learned and how the probabilistic inference is performed in the brain.

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Poster

743. Human Decision-Making: Risk, Effort, and Loss

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Topic: F.01. Human Cognition and Behavior

Support: NUS Grant WBS R-581-000-123-133

NUS Grant WBS R-581-000-133-112

Title: Risk preferences and choice strategies: Interrelationships across gains and losses domains

Authors: *O. A. MULLETTE-GILLMAN[1,5,2,3], Y. KURNIANINGSIH[4];
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Abstract: We investigated the relationships between individual risk preferences and choice strategies across the gains and losses domains during economic decision making. While prior theories and empirical studies suggest people are risk averse for gains and risk seeking for losses (Kahneman and Tversky, 1979, 2000), it is unclear whether preferences across domains are correlated - Are risk preferences in the losses domain a simple transform of preferences in the gains domain? Additionally, how does the option information that participants utilize in making their choices relate to their preferences? Here, we find that preferences are uncorrelated across domains, and that choice strategies, while correlated across domains, are correlated with risk preference in the losses domain, but not in the gains domain. Participants (N=50, 24 males, age = 23.4 ± 2.9 yrs) performed a two-alternative forced choice monetary decision task, with choices between certain and gamble options with varied absolute value and probability. Risk preferences for each domain were quantified through psychophysical indifference point analyses to produce domain-specific risk premium metrics. Choice strategy was quantified in each domain to determine the relative influence on choice behavior of two competing trial factors: 1) the relative expected value (rEV) of the options and 2) the probability of winning the gamble (pWin). In the gains domain participants were significantly risk averse ($t_{44} = 4.97$, $p < .01$). In the losses domain, individuals were significantly less averse than in the gains domain ($t_{43} = 3.70$, $p < .01$), but, contrary to prior theory were still, on average, weakly risk averse ($t_{47} = 2.95$, $p < .01$). Importantly, within participants there was no correlation between risk preferences across the gains and losses domains ($r = -.007$, $p = .96$). For choice strategies, across domains we found significantly higher use of the maximizing rEV information for losses than gains, with a significant correlation across domains ($r = .51$, $p < .01$). Interestingly, the relationship between preferences and choice strategies were differential across domains, with no significant relationship in gains ($r = .04$, $p = .78$), but a strong correlation in the losses domain ($r = -.62$, $p < .01$). The purpose of this study was to explicitly examine the interrelationships between preferences and strategies across domains within an intermixed task. Currently, with fMRI, we are leveraging this pattern of dissociable preferences (between domains) and choice strategies to preferences (within domains), to examine the neural architecture responsible for the value to utility transformations that are the basis of choice behavior.

Disclosures: O.A. Mullette-Gillman: None. **Y. Kurnianingsih:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

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Topic: F.01. Human Cognition and Behavior

Title: Synaptic substrates of cognitive biases during probabilistic inference

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Abstract: Most naturalistic decision making entails simultaneous learning and integration of information from various sources while the feedback is usually in a binary format (e.g. reward/no reward, correct/incorrect), requiring the brain to solve the nontrivial problem of assigning the correct weight to each piece of information. Recently, we have shown that computations necessary for performing probabilistic inference can be done at the synaptic level (Soltani and Wang 2010). However, it is not clear how much of the information stored in synapses, and reflected in the choice behavior, is consciously accessible to the subject and contribute to cognitive biases. In order to reveal synaptic substrates of cognitive biases, we expanded our previous computational model of probabilistic inference to simulate the experimental data from a probabilistic decision making task. On each trial subjects were presented with 4 cues and asked to make a decision between two alternative options in order to get point rewards. Subsequently, the subjects were asked to provide an estimate for how much each cue or a combination of cues predicts reward on one of the two targets (posterior probabilities). Experimental results showed that while subjects were able to closely estimate posteriors for individual cues or combination of cues, they also showed systematic but contradictory biases when priors were unequal. Namely, estimated posteriors for individual cues were biased toward the less rewarding outcome but posteriors for combination of cues were biased toward the more rewarding outcome, similarly to the choice behavior. Our model can explain the pattern of experimental results by assuming that information about each cue is stored in sets of plastic synapses that undergo dopamine-dependent learning, which is modulated by selective attention. Specifically, selective attention determines of the 4 cues presented on each trial, which ones activate corresponding neural populations and result in synaptic modifications (during learning and decision making), and which ones contribute to estimation. Overall, we show that not only a plausible learning rule at the synaptic level enables the model to perform probabilistic inference, but also explains the pattern of cognitive biases reported by human subjects.

Disclosures: **P. Khorsand:** A. Employment/Salary (full or part-time):; Jefferies LLC. **C. Sun:** None. **A. Soltani:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 743.05/RR49

Topic: F.01. Human Cognition and Behavior

Support: John Templeton Foundation Grant

Title: Humans tradeoff information seeking and randomness in explore-exploit decisions

Authors: *R. C. WILSON[1], J. D. COHEN[2];
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Abstract: The poster session you are presently attending is a giant explore-exploit dilemma. Do you exploit what you know and visit posters from people whose research you like, or do you explore contributions from people you don't know to discover something new? Exploiting is the surest way to see something good, but without exploring you might miss the very best results. Solving this dilemma optimally is intractable in all but the simplest settings, and so the question arises as to how humans balance exploration and exploitation in practice. In previous work we have shown that humans use at least of two strategies for solving the dilemma: a directed strategy in which choices are biased towards information, and a random strategy in which choice variability leads to exploration by chance. Directed exploration can be optimal but is hard to get right, while random exploration is suboptimal but simple to implement. In the present work we asked whether these two exploration strategies are used in a principled manner. That is, whether there is some kind of tradeoff between the optimality of directed exploration and the simplicity of random exploration. To do this we designed a task in which subjects made a series of choices between two options - an explore option and an exploit option - each paying out rewards between 1 and 100 points. Choosing the exploit option yielded a known outcome that was cued at the start of the trial. Choosing the explore option yielded an unknown outcome and the chance to increase the value of the exploit option on the NEXT trial. Specifically, if the random value of the explore option exceeded that of the exploit option, on the next trial the exploit option would take on that higher value, while the explore value would return to being random. This reward dynamic sets up a very simple explore-exploit dilemma that is amenable to theoretical analysis. We were thus able to show that there is indeed an optimal tradeoff between directed and random exploration in this task. Amazingly, this optimal tradeoff describes human performance remarkably well, with different people sitting at different points on the optimal tradeoff curve. Thus subjects that were more directed were less random, and subjects that were more random were less directed. These

results suggest that humans are able to tradeoff different types of exploration in a near-optimal manner to solve the explore-exploit dilemma.

Disclosures: **R.C. Wilson:** None. **J.D. Cohen:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 743.06/RR50

Topic: F.01. Human Cognition and Behavior

Support: Templeton Foundation Grant

Title: Risk, ambiguity and decision horizon in human exploration - A 'wheel of fortune' task

Authors: ***A. GEANA**, R. C. WILSON, J. D. COHEN;
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Abstract: Organisms are often faced with multiple resource alternatives, and must balance the tradeoff between pursuing known options (exploitation), and searching the environment for unknown opportunities (exploration). Exploration can be most beneficial in the presence of environmental uncertainty: when the range and benefits of all reward options are not fully known, exploration can lead to the discovery of new, better resources and an ultimately higher overall reward. Exploration can take two forms: random - an increase in decision noise that makes people less sensitive to the values of the available options, and directed - intentionally sampling unknown options to acquire information. Directed exploration is generally optimal, as it focuses on the options most likely to reveal new opportunities, but it is also computationally demanding. Random exploration is less efficient in acquiring information, but it is computationally more efficient. The exact mechanisms underlying these, and the factors that might lead an agent to choose one over the other, are unknown. We examined the influence of two types of uncertainty on people's exploration. One form of uncertainty, risk, is associated with choices in which possible outcomes and their probabilities are known, while the other, ambiguity, is associated with choices in which outcome probabilities, or even the outcomes themselves, are unknown. It is likely that people incorporate these two types of uncertainty differently into their decisions, which may bias them either against or in favor of exploration, and may influence the form that exploration takes. We used a 'wheel of fortune' task to separate risk and ambiguity, and examine how they impacted random and directed exploration. The

crucial manipulation involved the use of a decision horizon: the number of choices people were allowed to make under a given set of conditions before the environment changed. Longer decisions horizons incentivize exploration, as information acquisition is more useful than in shorter horizons. Our results suggest that, as predicted, the presence of ambiguity drives people to explore and acquire information; specifically, participants showed more exploration in long decision horizons. Interestingly, risk also appeared to impact exploration: participants showed a decrease in directed exploration as risk increased, while random exploration increased with higher risk levels. These findings indicate a potential trade-off between random and directed exploration, with more risky environments driving increased randomness and decreased information-seeking, while more ambiguous environments bias people toward information-seeking.

Disclosures: A. Geana: None. **R.C. Wilson:** None. **J.D. Cohen:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: Defense Science and Technology Agency Singapore (POD0713897)

National Medical Research Council Singapore (StaR/0004/2008)

Title: Neural anticipatory activity to expected value is correlated with loss aversion

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Abstract: Sensitivity to losses influences risky decision making. Here we identified brain networks sensitive to value and losses. During task-based fMRI, we offered twenty-nine healthy young adults (fifteen males, mean age 21.65 $\pm$ 2.42) years) 256 unique mixed gambles with equal chances of winning or losing amounts between $20 to $50 in $2 increments (de Martino et al, 2010). We selected a symmetric matrix for potential gains and losses to cover a large range of losses. Across trials, the overall expected value was zero. On average, participants showed loss aversion (mean "lambda" 1.21 $\pm$ 0.05), with choices more strongly driven by increasing loss amounts compared to gain amounts. Activity in left striatum was greater with gambles that were

later accepted compared to those that were rejected. Consistent with previous findings, the expected value of a gamble positively modulated BOLD activity in the anterior cingulate cortex (ACC) and bilateral inferior parietal lobe (IPL) (p<0.05 FWE corrected). Additionally, sensitivity to expected value correlated with loss aversion. Individuals who were more loss averse also evidenced stronger coupling between BOLD amplitude and expected value in the major hubs of the salience network namely the ACC and bilateral anterior insula (aINS). In the analysis of resting state fMRI data, seed-based analysis involving the ACC, IPL and aINS revealed that functional connectivity between left IPL and left aINS was negatively correlated with loss aversion (r=-0.62, p=0.0005 Bonferroni corrected). More loss-averse participants showed IPL resting activity that was more anti-correlated with activity in aINS. Our results demonstrate task-based and task-free functional networks sensitive to losses.

Disclosures: I.T. Kurniawan: None. **J. Tandi:** None. **M.W.L. Chee:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 743.08/SS2

Topic: F.01. Human Cognition and Behavior

Support: WT 086120/Z/08/Z

WT 096689/Z/11/Z

ERC ActSelectContext, 260424

BBSCR

Title: Behavioral and neural signatures of choice computation for human effort-based decision making

Authors: *S. BESTMANN[1], S. W. KENNERLEY[2], M. C. KLEIN-FLÜGGE[2];
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Abstract: Integrating costs and benefits is critical for optimal decision making. While much is known about decisions that involve abstract costs such as delay or uncertainty, little is known about how the brain evaluates choices requiring physical costs associated with actions. We used

a novel behavioral model which captures individual differences in effort discounting, together with functional magnetic resonance imaging in humans to identify brain regions that serve as a choice 'comparator' when choice options varied in their associated monetary reward and grip force. Decisions were temporally separated from action implementation, and presented in action or goods space, i.e. with or without knowledge of the associated motor responses. We demonstrate that in both cases, a region in the dorsal anterior cingulate cortex (ACC) fulfils the criteria for implementing the choice comparison; the difference between the chosen and unchosen options' reward and effort is coded positively and negatively, respectively, consistent with their effect on choice. Across subjects, the BOLD effect size for encoding subjective value difference specifically in ACC correlated with the influence value difference had on behavior, and thus carried behavioral relevance for choice. The location of these value difference activations within ACC overlapped with the rostral cingulate zone and ventral bank of the cingulate sulcus, and were thus distinct from medial frontal cortex and more anterior ACC where no signatures of choice computation could be identified. The putamen, a region anatomically interconnected with ACC and involved in action selection, switched from coding chosen-unchosen value difference at the time of choice to coding contralateral-ipsilateral value difference ('action value') at the time of response. This suggests the putamen may direct action selection towards the most valuable course of action. Previous work has shown that ventro-medial prefrontal cortex (vmPFC) compares rewards associated with abstract costs such as temporal delay or uncertainty. By contrast, here we failed to find any significant vmPFC responses with value but instead demonstrate that ACC compares rewards associated with effort and potentially other action-related costs. Furthermore, this role is independent of whether the resulting action is known at the time of choice, suggesting a general role for ACC in integrating information about effort into choice. Overall, our findings are consistent with electrophysiology and lesion studies suggesting that ACC plays a critical role in effort choices and that distinct neural circuits process decisions involving different types of costs.

Disclosures: S. Bestmann: None. **S.W. Kennerley:** None. **M.C. Klein-Flügge:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 743.09/SS3

Topic: F.01. Human Cognition and Behavior

Title: Decision making in three games with different amounts of risk and ambiguity

Authors: *B. ALBRECHT, U. KRAUS, P. MUSSEL, J. HEWIG;
Univ. of Würzburg, Würzburg, Germany

Abstract: Every day we make more or less risky financial, social or medical decisions. During the past decade various studies were conducted to describe how people react in such situations. Manifold experimental approaches were used differing in paradigms and concerning the parameters they integrated, e. g. one- or more-shot gambles, sure and risky options or only risky options, gambles with possible gains and losses or only with gains, usage of special tasks like the Iowa Gambling Task or Balloon Analogue Risk Task. Our intention for this study was to investigate how people behave under different levels of risk and uncertainty. We designed three different realistic risk games to simulate decision making in everyday life. The first one was a one-shot risk game with two options paired of high or low risk where possible gains and losses were integrated. The second one was an offer game where participants had to act as a manager who had to calculate an offer for different products which were described in a story. The decision screen then showed two possible gains with the associated probability of the acceptance for this offer. If the offer was rejected, the participant lost 20 Euro for the material and personal costs invested providing the offer. Finally, the third game was an investment game where participants had to choose between two amounts of money they invested in a stock. This investment stock game simulated decision-making under ambiguity. Here no probabilities were shown such as investing at the real stock market. 20 right-handed participants were measured with a 3 T fMRI scanner. In addition they had to answer different questionnaires about risk and ambiguity behavior and on personality traits. For the analysis of the fMRI measurements a parametric method was used with three conditions for the three different risk levels - high risk, low risk, and mixed risk (one high risk option and one low risk option paired). As parameters differences of possible gains and losses were added. For the fMRI results we found activity in the ACC, the VMPFC, the DLPFC, and in parietal regions. Decisions under ambiguity in the investment game compared to the other two games revealed higher activity in the ACC, the insula, and the inferior parietal lobe. Activation in occipital areas, regions of the parietal cortex, and the right IFG (BA 9) was decreased. For the other games activity in the lingual gyrus was higher during the risk game whereas activity in ACC, the insula, the DLPFC and middle frontal gyrus, the IFG, the inferior parietal lobe, and the precuneus were higher during the offer game. In conclusion, these imaging data results reveal the differences in the degree of uncertainty between the three risk games.

Disclosures: **B. Albrecht:** None. **U. Kraus:** None. **P. Mussel:** None. **J. Hewig:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

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Topic: F.01. Human Cognition and Behavior

Support: Alberta Gambling Research Institute

University of Alberta PER Human Performance Scholarship Fund

Title: The effect of described and experienced information on risky choice in a reaching task

Authors: *N. J. WISPINSKI[1], C. R. MADAN[2], C. S. CHAPMAN[3];
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Abstract: Risky decisions are often studied using options where information about risk and possible outcomes is explicitly described. However, in everyday life, information about risk is often learned through experiencing the outcomes directly. Here we investigated biases in risky decision-making when information was either described or experienced using both explicit (choice percentages and outcome frequency judgments) and implicit (reaction time and reach trajectory) measures. Participants reached to choose one of two shapes projected on a table while their movements were recorded. The selected shape gave participants either 0 or 10 points, and information about the probability of payoff for each shape (75%, 50%, or 25%) was either described (stated explicitly as a pie chart), or experienced (no probability information given, outcomes were learned through feedback). In phase 1 of the experiment, we tested how participants valued information by examining preferences when they were choosing between described and experienced options. Thus, throughout phase 1, participants learned about the experienced options. In the second half (phase 2), we tested preferences when participants chose between "old" experienced shapes (from phase 1) and "new" experienced options (only present in phase 2). When the experiment was complete, participants filled out a questionnaire to assess impulsive behavior and gambling-related cognition. Initial explorations of individual differences suggest that correlations between these personality measures and both explicit and implicit measures will be a valuable component to gaining a complete understanding of risky decision-making. While participants were accurate at estimating the outcome frequency for each shape, their choice percentages in phase 1 revealed a bias toward described shapes high in payoff probability and away from those low in payoff probability, relative to equivalent experienced shapes. In phase 2 we found a novelty-seeking choice preference toward "new" shapes that diminished over time as more experience with these shapes was accumulated. Interestingly, the dynamics of the reach (reaction time and movement trajectory) revealed results distinct from explicit probability estimates and choice behavior; reach dynamics were impacted by combinations of information type, as well as the expected value of a trial, consistent with enhanced reward processing. Overall, these findings demonstrate that how information about risk

is acquired, how the decision is physically made, and the individual preference profiles of participants are important, though often overlooked, components of risky decision-making.

Disclosures: **N.J. Wispinski:** None. **C.R. Madan:** None. **C.S. Chapman:** None.

Poster

743. Human Decision-Making: Risk, Effort, and Loss

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 743.11/SS5

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AA021529

Title: A novel fMRI delay discounting procedure to increase flexibility and minimize scanning duration

Authors: ***M. N. KOFFARNUS**, A. EKLUND, S. M. LACONTE, W. K. BICKEL;
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Abstract: Research on the rate at which people discount the value of future rewards has become increasingly prevalent as discount rate has been shown to be associated with many unhealthy patterns of behavior such as drug abuse, gambling, and overeating. fMRI research points to a fronto-parietal-limbic pathway that is active during decisions between smaller amounts of money now and larger amounts available after a delay. Researchers in this area have used different variants of delay discounting tasks and reported different contrasts between choice trials of different types from these tasks. For instance, researchers have compared 1) choices of delayed monetary amounts to choices of the immediate monetary amounts, 2) 'hard' choices made near one's point of indifference to 'easy' choices that require little thought, 3) trials where an immediate choice is available versus trials where one is unavailable, regardless of actual eventual choice, and 4) various combinations of the previous three trial types. These differences in procedure and analysis make it difficult to compare results across studies. In the present experiment, we designed a delay discounting task with the intended capability of being able to construct contrasts of all four comparisons listed above while optimizing scanning time to reduce costs and avoid participant fatigue. This was accomplished with an algorithm that customized the choice trials presented to each participant with the goal of equalizing choice trials of each type. We compared this task to two other delay discounting tasks previously reported in the literature (McClure et al., 2004; Amlung et al., 2012) in 24 participants. Results show that the novel task

yielded a similar activation pattern as the other two tasks when comparing similar contrasts and had at least as much power to resolve these differences per minute of scanning time. This novel task could be used in delay discounting fMRI studies to allow researchers to more easily compare their results to a majority of previous research while minimizing scanning duration.

Disclosures: **M.N. Koffarnus:** None. **A. Eklund:** None. **S.M. LaConte:** None. **W.K. Bickel:** None.

Poster

744. Attention: Individual Differences

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: NIDA Grant K01 DA027756

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Title: Can our thoughts change the amplitude of a TMS-evoked response?A preliminary study on the effect of visual and cognitive attention on TMS output

Authors: *S. BELL, M. S. GEORGE, C. A. HANLON;
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Abstract: Background: Transcranial magnetic stimulation (TMS) is an emerging therapeutic technique which is FDA approved for the treatment of depression and is being investigated as a potential therapy for addiction and pain. The 'dose' of TMS an individual receives is based upon their motor threshold (MT) - the minimum TMS current necessary to induce a motor evoked potential (MEP) 50% of the time. Although this is assumed to be a stable measurement for each individual, there is some evidence that the TMS-induced MEP can be influenced by the patient's visual and cognitive attention to the TMS-evoked response. The purpose of this study is to determine whether an individual can control the amplitude of the MEP through visual or cognitive attention. Method: Fourteen right-handed participants (ages 21-42, 9 Female, 5 Male) were recruited for the study. After locating the hand knob on the left primary motor cortex and determining resting MT, participants received 6 blocks of TMS pulses (120% MT, 20 single pulses per block). The blocks varied on the basis of visual attention (looking at the hand versus away from the hand), and cognitive attention (resting, actively inhibiting, or actively facilitating

the MEP). These conditions were randomized and counterbalanced across participants. Results: Two-way repeated measures ANOVA (visual attention x cognitive attention) revealed neither a significant interaction nor main effects of either vision or cognitive attention on MEP amplitude. This was true for both mean MEP amplitudes and percent changes of MEP amplitudes from rest across subjects. Conclusions: Overall these data demonstrate that participants are unable to consistently modulate their motor response to TMS through either active cognitive control or by simply looking at their hand. Future experiments with an increased sample size will allow us to investigate factors which may predict an individual's ability or inability to control MEP amplitude, such as confidence and mindfulness.

Disclosures: **S. Bell:** None. **M.S. George:** None. **C.A. Hanlon:** None.

Poster

744. Attention: Individual Differences

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Support: CIHR Grant MOP-130361

Title: Morphology and patterns of the posterior intermediate parietal sulcus in the parietal lobe of the human brain

Authors: *V. ZLATKINA, M. PETRIDES;
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Abstract: The angular gyrus is a functionally-heteromodal region located in the posterior part of the inferior parietal lobe of the human brain and it is involved in language, mathematical operations and visuospatial processing. The posterior intermediate parietal sulcus emerges out of the inferior bank of the intraparietal sulcal complex, consisting of the intraparietal and paroccipital sulci, and courses between the second and third caudal terminations of the superior temporal sulcus in the angular gyrus. The relationship between the posterior intermediate parietal sulcus and the intraparietal sulcal complex is poorly understood and requires clarification. The posterior intermediate parietal sulcus was examined systematically in serial sections of forty Magnetic Resonance Imaging (MRI) brain volumes registered in the Montreal Neurological Institute (MNI) proportional stereotaxic space. The results showed that in a significant number of cases, the posterior intermediate parietal sulcus was formed by downward side-branches of the

intraparietal and paroccipital sulci, which were either well-developed or poorly-developed, in which case they barely indented the inferior banks of the intraparietal and paroccipital sulci. In a number of cases, the posterior intermediate parietal sulcus was formed by a short sulcus, which associated with the paroccipital sulcus via a gyral passage. In some hemispheres, the posterior intermediate parietal sulcus merged with the caudal branches of the superior temporal sulcus on the surface of the brain. Knowledge of the specific morphological patterns of the posterior intermediate parietal sulcus with the neighbouring sulci would allow a detailed study of the structure-to-function relations in the region of angular gyrus.

Disclosures: V. Zlatkina: None. **M. Petrides:** None.

Poster

744. Attention: Individual Differences

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Topic: F.01. Human Cognition and Behavior

Support: NIH/NIA/AG030770

Title: Resting-state anticorrelations between medial and lateral prefrontal cortex: Association with working memory, aging, and individual differences

Authors: *J. B. KELLER[1], T. HEDDEN[2], T. W. THOMPSON[1], S. A. ANTERAPER[1], J. D. E. GABRIELI[3,4], S. WHITFIELD-GABRIELI[1];
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Abstract: We examined how variation in working memory capacity due to aging or individual differences among young is associated with intrinsic or resting-state anticorrelations, particularly between (1) the medial prefrontal cortex (MPFC), a component of the default-mode network that typically decreases in activation during external, attention-demanding tasks, and (2) the dorsolateral prefrontal cortex (DLPFC), a component of the fronto-parietal control network that supports executive functions and working memory and typically increases in activation during attention-demanding tasks. We compared the magnitudes of MPFC-DLPFC anticorrelation between healthy younger and older participants (Experiment 1) and related the magnitudes of

these anticorrelations to individual differences on two behavioral measures of working memory capacity in two independent groups of young adults (Experiments 1 and 2). Relative to younger adults, older adults exhibited reductions in working memory capacity, in MPFC-DLPFC anticorrelations, and in gray matter density in the MPFC. Within younger adults, greater MPFC-DLPFC anticorrelation at rest correlated with greater working memory capacity. Theses findings show that variation in MPFC-DLPFC anticorrelations, whether related to aging or to individual differences, may reflect an intrinsic functional brain architecture supportive of working memory capacity.

Disclosures: J.B. Keller: None. **T. Hedden:** None. **S. Whitfield-Gabrieli:** None. **T.W. Thompson:** None. **S.A. Anteraper:** None. **J.D.E. Gabrieli:** None.

Poster

744. Attention: Individual Differences

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Topic: F.01. Human Cognition and Behavior

Support: ICB Grant ICBHM1

Title: Individual differences in relative-heading performance

Authors: *H. BURTE[1], M. HEGARTY[2], B. O. TURNER[2], M. B. MILLER[2];
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Abstract: Individuals differ greatly in their ability to learn new environments and to travel effectively through known environments, but the neural basis of these differences is unclear (Sholl, Kenny, & DellaPorta, 2006). The presubiculum has been identified as responsible for one aspect of effective navigation, namely, identifying allocentric-headings (Vass & Epstein, 2013). Allocentric-headings are facing directions that are fixed to the external environment, such as the cardinal directions. However, the ability to identify allocentric-headings from photographs has shown significant individual and strategy differences (Burte & Hegarty, 2014). To determine the contribution of individual differences on the ability to identify allocentric-headings, participants completed a battery tests on navigation preferences and spatial abilities, completed a Relative-Heading task within fMRI, and completed a strategy questionnaire. The Relative-Heading task consisted of an imagined heading presented by text, indicating a heading within a familiar environment that the participant imagined facing. Imagined headings used large-scale spatial

referents from the local environment, such as a mountain range. Participants were then presented with a photograph of a building from within the familiar environment (or picture heading) and responded with the difference in heading between the imagined and pictured headings. Task performance was significantly correlated with self-assessed sense-of-direction, perspective-taking ability, and right-left discrimination ability. Using Representational Similarity Analysis (RSA), individual differences in directional coding of imagined and pictured headings were assessed within the presubiculum and retrosplenial region. The neural bases of strategy differences were assessed via activation in medial parietal and medial temporal lobes, based on the strategies participants reported using. By identifying the underlying neural bases of individual differences within directional abilities, this research provides insights into the neural bases of the large differences within human navigational abilities.

Disclosures: H. Burte: None. **M. Hegarty:** None. **B.O. Turner:** None. **M.B. Miller:** None.

Poster

744. Attention: Individual Differences

Location: Halls A-C

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Program#/Poster#: 744.05/SS10

Topic: F.01. Human Cognition and Behavior

Title: Neural correlates of verbal creativity: Magnetic resonance morphometric analysis

Authors: S. A. KOZLOVSKIY[1], M. M. PYASIK[1], A. V. VARTANOV[1], *V. L. USHAKOV[2]; [1]Fac. of Psychology, Lomonosov Moscow State Univ., Moscow, Russian Federation; [2]NRC Kurchatov Inst., Moscow, Russian Federation

Abstract: Objective: Present study investigates the role of hippocampus and cingulate cortex regions in various verbal creativity characteristics in healthy aging. **Methods:** Subjects: 14 right-handed females (59-85 years old, mean age - 70.5) with no neurological or psychological disorders. We performed magnetic resonance morphometric analysis of T1 images to measure absolute square surfaces (in mm^2) of three cingulate cortex regions - anterior (BA 24), posterior ventral (BA 23), posterior dorsal (BA 31) areas, and hippocampi volume (in mm^3) in both hemispheres. Verbal creativity was measured with the Russian adaptation of Guilford's Alternate Uses test (Guilford 1967; Averina & Scheblanova, 1996), which allows to assess the following creativity characteristics: originality, fluency, flexibility and overall creativity. We calculated non-parametric correlations (Spearman coefficient, $p < .05$) between creativity characteristics and morphometrical data. **Results:** Overall verbal creativity correlates positively with the size of

right posterior ventral cingulate cortex ($r = .734$, $p < .01$) and negatively with the size of right anterior cingulate cortex ($r = -.560$). Higher originality ($r = .783$, $p < .01$) and flexibility ($r = .595$) scores correlate with the increased size of right posterior ventral cingulate cortex. Moreover, fluency correlates positively with the size of the same cingulate cortex region ($r = .598$) and the volumes of both left ($r = .568$) and right ($r = .587$) hippocampi. No significant correlations between other cingulate cortex regions and creativity characteristics were revealed. **Discussion and conclusions:** It is known that hippocampus is involved in associative memory processes and the volume of left hippocampus is related to the person's vocabulary (Jung et al., 2014). Consequently, its weaker functioning results in worse activation of associative connections, which can explain lower fluency of creativity. Posterior ventral cingulate cortex is connected to hippocampus, as well as anterior cingulate cortex and precuneus (related to emotional evaluation), which can explain the relation of this brain region to overall verbal creativity scores, along with originality, fluency and flexibility. Anterior cingulate cortex is connected to prefrontal cortex and is known to be the 'error detector' (Kerns et al., 2004). This can explain why the increased size of this brain structure is related to the decrease in overall creativity. Correlations between various creativity characteristics and the right hemisphere brain structures can be related to the fact that producing original answers may require imagination, rather than verbal thinking.

Disclosures: S.A. Kozlovskiy: None. **M.M. Pyasik:** None. **A.V. Vartanov:** None. **V.L. Ushakov:** None.

Poster

744. Attention: Individual Differences

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 744.06/SS11

Topic: F.01. Human Cognition and Behavior

Support: TOYOTA, Japan

Title: Phase locking of motor and parietal cortices is associated with the individual differences in early visuomotor adaptation

Authors: *M. ANWAR[1,2], Y. NAKAGAWA[2], K. KITAJO[2];
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Abstract: People differ in their ability to learn a new motor skill and as a result some people perform motor activities better than the others. The functional MRI studies showed individual motor learning was correlated with greater functional activation in the prefrontal, premotor, and parietal cortices. So, it is possible that the activation variations of specific brain regions across the population influence motor skill learning. This electroencephalogram (EEG) and motor skill adaptation based study is aimed at investigating how brain networks are different in slow and fast learners during early stage of adaptation. A total of fourteen subjects participated in a visumotor adaptation task. The subjects performed goal directed reaching out movements in a visually perturbed environment. The rate of adaptation to the perturbed environment was calculated as ratio between errors in non-perturbed movements and errors during perturbed movements. Multi-channel EEG (64 channels) was also recorded during the adaptation task and was stored for offline analysis. We calculated phase locking factor (PLF) for each subject across first 48 trials within one electrode. The PLF was adjusted to the base line (-1s to -0.5s of target onset) to calculate PLFz. The PLFz value was then compared with individual's visuomotor adaptation rate for r-square statistics. We found higher theta band PLFz value at P3 (left parietal cortex, $r2 : 0.32$ $p<0.02$), P2 (right parietal cortex, $r2 : 0.28$ $p<0.03$) and alpha band PLFz at C3 (left motor cortex, $r2 : 0.45$ $p<0.01$) for individuals with higher rate of adaptation. These findings suggest that the people who perform better in adapting a perturbed environment show strong intert-trial phase locking at parietal and motor cortices. This study will help us understanding the individual differences in brain activity during the early stage of adaptation. As a result more effective and targeted neuro-feedback techniques could be developed to overcome performance deficits, to acquire new skills, and to enhance performance of well-learned skills.

Disclosures: **M. Anwar:** Other; TOYOTA, Japan. **Y. Nakagawa:** Other; TOYOTA, Japan. **K. Kitajo:** Other; TOYOTA, Japan.

Poster

744. Attention: Individual Differences

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 744.07/SS12

Topic: F.01. Human Cognition and Behavior

Title: Sex specific relationships between inner speech and schizotypal personality traits

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Abstract: Inner speech plays a role in the rehearsal process of verbal working memory (Baddeley, 1992), planning (Lidstone, et al., 2010), and self-awareness (Morin & Michaud, 2007). Models of auditory verbal hallucinations (AVH) link to misidentification of inner speech in those diagnosed with schizophrenia. Concordantly, schizophrenia patients and their first-degree family members have trouble recognizing their own face compared to controls (Irani, et al., 2006). People with traits in common with schizotypal personality disorder show similar patterns to people with schizophrenia (Platek, et al., 2003). Despite its role in verbal and working memory and its role in AVH, little has been done to qualitatively describe inner speech. Furthermore, no study has investigated the relationship between dimensions of inner speech and working memory nor between these dimensions and variations in schizotypal traits in a typical population. Participants (n = 52, 58.8% female) were students between the ages of 18 and 21, at a small, private university in northeastern Pennsylvania. Participants were assessed for inner speech qualities and schizotypal personality traits the Varieties in Inner Speech Questionnaire (VISQ) and the Schizotypal Personality Questionnaire (SPQ), respectively. Participants also completed a variety of cognitive tasks, including mental rotations and tests of memory for words, faces, and shapes. There was a significant correlation between the degree to which inner speech is dialogic and mental rotations score for males ($r(19) = .44$, $p = .046$), but not for females ($r(28) = -.302$, $p = .105$). Overall SPQ scores was significantly correlated with the degree to which inner speech is dialogic ($r(49) = .365$, $p = .008$). The cognitive-perceptual factor of the SPQ was significantly correlated with the evaluative-motivational factor of the VISQ for all participants ($r(49) = .367$, $p = .008$). This correlation seemed to be driven by females ($r(28) = .458$, $p = .011$), not males ($r(19) = .212$, $p > .05$). The interpersonal factor of the SPQ was significantly negatively correlated with condensed inner speech ($r(49) = -.315$, $p = .024$). This correlation was also driven by females ($r(28) = -.468$, $p = .009$), not males ($r(19) = -.107$, $p > .05$). Overall, variation in inner speech seems related to performance on mental rotations tasks and to schizotypal personality traits. The relationship between inner speech and schizotypal personality traits appears to be sex-specific, as evidenced by the different relationships between inner speech and traits measured by the SPQ in males and females.

Disclosures: **B. Zimmerman:** None. **C.E. Lowe:** None. **J. Cannon:** None. **P.T. Orr:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1-ZIA-MH002794-12

Title: Covariance between right orbitofrontal and paralimbic cortical thickness is associated with antisocial traits in typically developing youth

Authors: *G. L. WALLACE[1], N. LEE[2], A. RAZNAHAN[2], L. CLASEN[2], A. MARTIN[2], J. GIEDD[2];
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Abstract: Prior research suggests that behaviors associated with antisociality can be viewed as dimensional wherein clinical disorders lie at the extreme of a continuous distribution of these quantitative traits. Because these behaviors are continuous and extend into the general population, we can seek to identify neural endophenotypes in relatively unconfounded (i.e., no comorbidities) subclinical samples. To this end, we sought to examine neural correlates of antisocial traits in typically developing youth. The neural correlate of interest in the current study was covariance of cortical thickness (i.e., how correlated thickness is in each part of the cortical sheet with every other part of the cortical sheet), because this approach has been shown to provide overlapping yet unique information from connectivity metrics utilized in white matter quantification and functional MRI. Utilizing the Antisocial Process Screening Device, parent or self-ratings of antisocial traits, including callous-unemotional traits were obtained from 179 typically developing youth. All participants also provided one 1.5 Tesla structural MRI scan from which high-resolution, vertex-based cortical thickness metrics were derived using the CIVET pipeline. An approach termed Mapping Anatomical Correlations Across Cerebral Cortex (MACACC) was used so that cortical thickness at each of ~80,000 vertices was correlated with mean cortical thickness across the entire cortical sheet as a function of antisocial trait ratings. With increasing antisocial traits, there was an increased ($q<.05$) association between the thickness of the right orbitofrontal cortex and mean cortical thickness (i.e., average thickness of ~80,000 vertices across the cortical sheet). The apparent driving force behind this association was increased coupling between right orbitofrontal cortex and a discrete 'network' of regions, composed primarily of paralimbic cortex, for youth with fewer antisocial traits. In contrast, more diffuse correlations between right orbitofrontal cortex and the rest of cortex was found among those with higher antisocial trait ratings. The same pattern of findings was observed for callous-unemotional traits. Lower antisocial trait ratings are associated with a more specific cortical thickness covariance structure (with higher correlations) involving the right orbitofrontal and paralimbic cortices. These individual differences results within a sample of typically developing youth closely parallel not only prior findings of dysfunctional white matter connectivity in antisocial groups but also prominent models of neural dysfunction proposed to underlie antisocial behavior.

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Poster

744. Attention: Individual Differences

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Program#/Poster#: 744.09/SS14

Topic: F.01. Human Cognition and Behavior

Title: Increased peak gamma frequency induced by visual stimuli in those with high levels of autistic traits

Authors: *A. DICKINSON, M. JONES, M. BRUYNS-HAYLETT, E. MILNE;
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Abstract: In addition to differences in social interaction and communication, individuals with autism spectrum disorder (ASD) often show atypical sensory perception. Both enhancements and impairments in discrimination thresholds have been found across several sensory modalities in ASD. In the visual domain it has been found that both individuals with autism (Bertone et al., 2005) and those with a high amount of autistic traits (Dickinson, Jones & Milne, submitted) demonstrate superior orientation discrimination thresholds. One explanation put forward for the atypical discrimination thresholds in ASD is that atypical neural connectivity may lead to an increased amount of lateral inhibition (Bertone et al., 2005). However, others have suggested that there may be an increased ratio of excitation to inhibition in the brains of those with ASD (Rubenstein & Merzenich, 2003). Measuring the frequency at which gamma activity reaches its peak amplitude provides a way for us to investigate the relationship between orientation discrimination, autistic personality traits and neural activity. Peak gamma frequency is said to be a marker of the balance between neural excitation and inhibition (Brunel and Wang, 2003) and has previously been found to be correlated with orientation discrimination thresholds and levels of the inhibitory neurotransmitter, GABA (Edden et al, 2009). This study aimed to see whether autistic personality traits, orientation discrimination thresholds and peak gamma frequency were related in a neurotypical sample (N=33). We used a psychophysical task to measure orientation discrimination, along with a separate session during which we recorded electroencephalography whilst participants viewed a grating to measure visually induced gamma activity. We found a significant relationship between orientation discrimination thresholds and level of autistic personality traits ($r=-.492$, $p=.004$). We also found a significant relationship between peak

gamma frequency and both orientation discrimination thresholds (r=-.544, p=.001) and level of autistic personality traits (r=.489, p=.004). These results suggest that individuals with a higher level of autistic personality traits show both lower orientation discrimination thresholds and higher peak gamma frequency. Our results therefore provide further evidence that there might be an imbalance in levels of excitation and inhibition associated with the presence of autistic personality traits. In light of previous work, we put forward that in addition to mediating peak gamma frequency and orientation discrimination, GABA levels may be associated with autistic traits in the general population.

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Poster

744. Attention: Individual Differences

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Title: Deriving core psychiatric behavioral constructs and their neural correlates

Authors: E. T. MARCELLE[1], E. J. HO[1], D. J. LURIE[1], D. O'CONNOR[1], Z. SHEHZAD[2,1,3], R. CRADDOCK[1,3], R. H. TOBE[3], F. X. CASTELLANOS[3,4], B. L. LEVENTHAL[3], S. J. COLCOMBE[3], *M. P. MILHAM[1,3];
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Univ., New Haven, CT; [3]Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY; [4]NYU Child Study Ctr., NYU Ctr. Neurodevelopmental Disorders, New York, NY

Abstract: As psychiatry works to move from a categorical, syndrome-based perspective of psychiatric illness to one based upon neuroscience, a key challenge is to identify core behavioral constructs and their neurobiological correlates. In this regard, the present work attempts to provide a model by data-driven analysis that can be used to identify psychiatrically-relevant behavioral constructs and map them to variations in brain function across individuals. To accomplish this goal, we first applied exploratory factor analysis (EFA) to a battery of dimensional questionnaires designed to probe various psychiatric domains, and then related these factors to an array of commonly measured resting-state fMRI (R-fMRI) indices of human brain function. Data for this effort was collected as part of the ongoing Nathan Kline Institute-Rockland Sample initiative, which is acquiring phenotypically rich, multimodal imaging datasets from community-ascertained individuals ages 6.0-85.0 years old (approximately half of which have a lifetime history of one or more DSM-based psychiatric illnesses). The present work limited its focus to 288 participants aged 18-59. Phenotypic data included 16 self-report questionnaires assessing a wide range of behavioral functioning including but not limited to: impulsivity and risk-taking behavior; posttraumatic symptomatology; attention-deficit and hyperactivity symptoms; temperament and personality; depression and anxiety; empathic, callous, and unemotional traits; cognitive failures; and adaptive functioning. Participants also completed an abbreviated intelligence testing, as well as an MRI session that included a R-fMRI scan (10 min, TR=1400ms, voxel size=2mm isotropic). EFA (principal axis factoring, oblimin rotation) revealed 5 factors, which were interpreted as: emotional dysregulation, impulse control, sociability, lifestyle factors, and mindfulness. R-fMRI indices examined included: fractional ALFF (fALFF), Regional Homogeneity (ReHo), and Voxel-mirrored Homotopic Connectivity (VMHC). For each factor, we found a distinct R-fMRI profile across the various measures included. Of particular note was mindfulness factor, which was associated with the dorsal caudate, nucleus accumbens and brainstem. These findings are consistent with existing literature relating striatal function to attentional control.

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Poster

744. Attention: Individual Differences

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Topic: F.01. Human Cognition and Behavior

Support: NIH F31AG047037

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Title: Trait mindfulness is associated with resting state functional connectivity of the default mode network

Authors: *C. M. STILLMAN[1], X. YOU[2], E. RASMUSSEN[2], C. J. VAIDYA[2], R. S. TURNER[3], J. H. HOWARD, Jr.[4], D. V. HOWARD[2];
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Abstract: Mindfulness refers to our ability to maintain awareness to internal and external phenomena and is associated with many health benefits. The default mode network (DMN) is a critical functional network of the brain and includes regions more active during resting than task states and also when thinking about oneself than others. This network dissociates into two subsystems that interact via a midline "core" comprised of the posterior cingulate (PCC) and anterior medial prefrontal (aMPFC) cortices (Andrews-Hanna et al., 2010). The dorsomedial prefrontal cortex (dMPFC) subsystem is more active during present-oriented thinking, and the medial temporal lobe (MTL) system during future-oriented thinking. Both subsystems and the core are more active during internally- than externally-oriented cognition, leading to the possibility that better integration of the network as a whole may promote internal awareness. Given that mindfulness has also been linked to greater internal awareness, we predicted that people higher in trait mindfulness would have stronger resting state functional connectivity (rsFC) between the DMN core and nodes from both subsystems. Thirty adults (ages 18-27) completed the Mindful Attention Awareness Scale (MAAS) as part of a neuroimaging study. Higher scores on the MAAS indicate higher trait mindfulness. Participants were then scanned in the resting state with eyes open for 11 minutes. An aMPFC seed was chosen based on functional networks identified in a demographically similar sample (Gordon et al., 2012). This seed corresponded well to the aMPFC "core" node identified by Andrews-Hanna et al. Seed-based voxel-wise analyses were conducted in SPM8 to generate connectivity maps of the aMPFC for each subject. These maps were entered into a regression with MAAS scores entered as the covariate of interest and mean framewise displacement as a covariate of no interest. As predicted, higher mindfulness was associated with stronger rsFC to nodes in the core and MTL subsystem of the DMN (PCC extending into retrosplenial cortex and right angular gyrus; $p < 0.005$, $k > 20$, uncorrected). However, there were no positive relationships with the dMPFC system. Higher mindfulness was also associated with weaker rsFC to regions of the executive control network (caudate and middle frontal gyrus) often engaged during processing of external

stimuli. Perhaps stronger within-network integration of regions associated with internal processing and future orientation, and stronger disintegration with external processing regions enable the better adaptive skills that have been linked to mindfulness.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: CREB1 genotype effects on individual differences in the adaptation of reward-motivated decisions and related activation of mesocorticolimbic regions in humans

Authors: C. WOLF[1,3], H. MOHR[1,4], E. DIEKHOF[2,5], M. KEIL[2], E. BINDER[6,7], *O. GRUBER[8];
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Abstract: The cyclic AMP response element binding protein (CREB) acts as an important adapter of mesocorticolimbic networks through its impact on activity-regulated transcription and plasticity in neurons. Activity or expression changes of CREB in mesocorticolimbic regions such as the nucleus accumbens (NAc) and orbital frontal cortex (OFC) interact with behavioral changes during reward-motivated learning. Neurons in these regions are CREB activity sensitive and neural activation of these brain areas is critically involved during reward-motivated behavior. This evidence is based on animal models whereas the influence of CREB on mesocorticolimbic regions or reward-associated learning has not been investigated in humans. We addressed this question by testing whether genetic variations of the *CREB1* gene contribute to individual differences in the adaptation of reward-motivated decisions and related brain activation, using BOLD-fMRI in 224 young and healthy students. During the experiment

participants needed to adapt their decision to either pursue (Desire) or resist (Reason) immediate rewards to optimize the delayed reward outcome. We probed for genotype effects by conducting analyses of behavioral data, whole-brain as well as a priori region of interest based imaging data. Our analysis revealed that *CREB1* genotype effects significantly contribute to individual differences in choices when pursuing immediate rewards to increase the delayed reward outcome (Desire) and the absence of such effects when resisting immediate rewards to maintain fixed rewards (Reason). The same genetic variation also significantly modulated the BOLD-signal in the NAc, OFC, insula cortex, cingulate gyrus, hippocampus, amygdala and precuneus during decisions pursuing increased in comparison to those pursuing fixed rewards. Our converging behavioral and imaging results support the view that similar relations between reward- and associative learning-related behaviors and the neuronal activation and/or expression of CREB in these regions exist in animals and humans. *CREB1* genotype effects on mesocorticolimbic areas thus could contribute to individual differences in reward- and associative memory-based decision making.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: R01 AG030311

Title: Hippocampal connectivity to posterior cingulate cortex reliably predicts memory across sessions and age groups

Authors: *A. TOUROUTOGLOU[1,2], J. ANDREANO[1], M. ADEBAYO[1], M. STEPANOVIC[1], C. CASO[1,2], L. FELDMAN BARRETT[1,3], B. DICKERSON[1,2];
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Abstract: BACKGROUND: Despite the optimistic results of initial intrinsic connectivity reliability studies, no study to our knowledge has sought to investigate whether intrinsic

connectivity-behavioral relationships are stable over time. The possibility that these relationships may be stable traits could be addressed by using data from one point in time to predict relationships at a different time point within the same individuals. We investigated the stability of such brain-behavior relationships at two timepoints, approximately 1 week apart, testing the hypothesis that intrinsic connectivity at one point in time would predict behavior at a later point in time, and vice versa. METHODS: We focused on an individual differences brain-behavior finding previously reported by our laboratory (Wang et al. (2010a): episodic memory performance of older adults was predicted by the strength of hippocampal to posterior cingulate/precuneus cortex (PCC) connectivity. We analyzed behavioral and resting state scan data on forty-one adults (mean age = 28.07, SD= 9.51; 23 females) collected at two timepoints, approximately 1 week apart. To examine memory performance, we computed discriminability on a paired associate recognition memory task. To examine the intrinsic functional connectivity strength between the hippocampus and PCC, we used seed-based resting state functional connectivity MRI analysis. Using a series of linear regression analyses, we next examined the relationship between hippocampal connectivity to PCC and memory performance at two timepoints. RESULTS: Our results showed that the same relationship observed in Wang et al. (2010)'s study generalizes to an independent sample of participants of a different age, and further that the relationship within this sample is reliable over two points in time, such that connectivity at either timepoint predicts memory performance during the other timepoint. CONCLUSIONS: These findings provide the first evidence that the relationship between large-scale intrinsic network connectivity and episodic memory performance is a stable trait that varies between individuals. Critically, intrinsic connectivity between the hippocampus and PCC significantly predicts performance on a memory task one week later. Our results thus suggest that the functional connectivity between hippocampus and PCC is a trait-level characteristic which predicts associative learning ability, rather than a transient state.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NRF-2012-0006587

Title: Individual difference in individualism/collectivism modulates functional connectivity between medial prefrontal cortex and posterior cingulate cortex during resting state

Authors: *Y. BAK, K.-J. TARK, H. KIM, D. YI;
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Abstract: One of fundamental questions in social neuroscience is how the brain represents the self in its cultural context. The cortical midline structures (CMS) including medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC) have been implicated in the representation of the self. Many of recent neuroimaging studies have targeted to reveal the cultural influences on the activations in these regions. It remains unclear, however, how functional coupling between these sub-regions of CMS reflects individuals' cultural orientation during resting state. Using functional magnetic resonance imaging (fMRI), we previously reported how mPFC and PCC interact with each other in a self-referential task (Bak, Tark, Kim, & Yi, 2014, Cognitive Neuroscience Society Annual Meeting). Before scanning, we primed participants with either individualistic or collectivistic construal. In each trial during scanning, participants viewed a trait adjective and judged how well it described a given person (referent: self vs. president) from a given point of view (viewpoint: own view vs. friend's view). At least a day after scanning, participants completed self-construal scale (Singelis et al., 1995). As results, we found that the psychophysiological interaction is enhanced between mPFC and PCC during self-referential processing, and that two-way interaction in PCC was significantly modulated with individuals' individualism/collectivism bias. However, we could not find any effects of priming either in mPFC, PCC, or their functional connectivity. These results suggest us that functional coupling between mPFC and PCC might be rather stable over time and less subject to priming manipulation. Our finding led us to hypothesize that individual difference in individualism/collectivism bias might be reflected in the functional connectivity between mPFC and PCC during resting state. Participants (N=27) rested for 6 minutes in the scanner without any task. After scanning, they completed self-construal scale. Resting-state functional data were analyzed to construct correlational coefficient maps for each participant by calculating the correlation of the BOLD time series in the 6-mm-radius mPFC seed and the remaining voxels in the whole brain. As a result, we found that the mPFC functional connectivity with PCC is significantly modulated with individuals' individualism/collectivism bias. The less individualistic an individual was, the greater connectivity between mPFC and PCC was observed. Overall, our finding suggests that cultural variations in self-construal might be engraved as long-term patterns of functional connectivity in CMS.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 25350994

Title: Personality traits are carved in the resting-state brain networks

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Abstract: It has been suggested that a number of brain regions are involved in personality traits; however, their neural correlates remain largely to be established. Further understanding of the underlying neural basis would provide better insight into personality disorders. In this study, we examined the relationship of personality estimates (obtained from Cloninger's Temperament and Character Inventory, TCI) with a voxel-wise measure of global functional connectivity ("regional global connectivity," rGC) as measured by resting-state functional magnetic resonance imaging (MRI) (Ueyama et al., PLoS ONE, 2013). Japanese version of TCI, consisting of a total of 240 yes/no questions, was applied to subjects on the day of image acquisition. Big-Five scores were estimated by TCI scores. We used a 3-Tesla MRI (PHILIPS) to obtain T1-weighted structural and functional images from healthy right-handed (Edinburgh Handedness Inventory score > +70) male subjects (N=89, 18-24 years old). For acquisition of functional images, we used a gradient-echo echo-planar pulse sequence (TR = 3000 ms) sensitive to the blood oxygenation level dependent (BOLD) contrast. Subjects were asked to stay awake with their eyes closed during acquisition (a series of three 5-min sessions). Preprocessing of BOLD signal was performed through SPM8 and in-house software developed on MATLAB (Mathworks): head motion realignment, normalization with the International Consortium for Brain Mapping Echo-Planar Imaging template, spatial/temporal smoothing and noise reduction. For each voxel (down sized to 6x6x6 mm) in the gray matter, cross-correlation coefficients with all other voxels (i.e., functional connectivity) were derived and averaged to determine an rGC value. We found significant positive correlations between 5 of 7 TCI scores (Novelty Seeking, Reward Dependence, Persistence, Self-Directedness and Self-Transcendence) and rGC, revealed by Pearson's linear correlation coefficient ($p<0.05$, corrected for multi-comparison with Monte Carlo simulation obtained from AlphaSim), at distinct gray matter regions, not only in the cerebral cortex such as dorsolateral prefrontal cortex, orbitofrontal cortex and occipital-parietal cortex, but also in subcortical structures such as caudate nucleus and cerebellar hemisphere. Significant negative correlations were found between Cooperativeness score and rGC at precuneus. We also found that 4 of Big-Five scores were related to the rGC in the specific brain

regions. Our results suggest that the personality traits are represented in the resting-state brain networks that involve specific cortical and subcortical regions related to each trait.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Undergraduate Research Fellowship from the University of Texas

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Title: The relationship between social media use, circadian rhythms, and mood regulation in young adults

Authors: ***S. WITKOWSKI**[1], K. KALLINA[2], C. KARR[3], S. SHERMAN[2], D. SCHNYER[2]; [1]Psychology, [2]Univ. of Texas, Austin, TX; [3]Northwestern Univ., Evanston, IL

Abstract: College students exhibit variations in circadian rhythms that can lead to poor sleep quality and decreased psychological health. Circadian rhythms (CR) are 24-hour cycles generated by the suprachiasmatic nucleus. Previous work suggests that irregular CRs are associated with lower cognitive performance and negative mood. A number of behaviors have also been linked to mood, including the use of social media (i.e., online social networking sites, texting, electronic communication), but its relationship to suboptimal sleep remains unclear. The purpose of this study was to examine the relationship between circadian rhythms, mood, and social media usage in young adults. Twenty-six college-aged adults were recruited to participate in a ten-day study. An android smartphone application created by the Center for Behavior Technologies at Northwestern University tracked daily social media use, texting frequency and mood across the 10-day period. Along with monitoring of social media use, sleep/activity patterns were recorded using actigraphy and verified against participant-completed daily sleep logs. Daily mood values were averaged across the ten-days and total time spent on social media was summed for each participant. Actigraph data was used to calculate CR measures in order to characterize the frequency and stability of activity. Less stable sleep/activity patterns were correlated with decreased overall average mood ratings and greater total social media usage. The

results of this study reveal a clear relationship between social media use and CR patterns and possibly support a model of sleep providing a moderating role between social media use and mood. Our ongoing investigation of additional sleep/activity measures could provide further insight into the relationships among sleep, mood, and social media usage. This study is the first to demonstrate the utility of tracking real time social interactions through the use of a smart phone across a multi-day period.

Disclosures: S. Witkowski: None. **K. Kallina:** None. **S. Sherman:** None. **D. Schnyer:** None. **C. Karr:** None.

Poster

744. Attention: Individual Differences

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 744.17/SS22

Topic: F.01. Human Cognition and Behavior

Title: Estimating sleep, mood and time of day effects in a large database of human cognitive performance

Authors: *D. A. STERNBERG, K. KATOVICH, J. L. HARDY, M. SCANLON;
Lumos Labs, San Francisco, CA

Abstract: Acute intra-individual factors such as sleep, mood, and time of day can have a meaningful impact on cognitive performance, but understanding these relationships depends on access to a sufficiently large dataset with sufficient variability. In this analysis, we applied Lumosity's growing database of human cognitive performance to these problems by combining available data about the time of day users trained and their demographic profile with additional information gleaned from a new survey feature that was recently tested as part of a web-based cognitive training platform. Before beginning each daily workout, Lumosity users were prompted to enter information about their sleep (in one hour increments from "≤5" to "9+") and mood (very negative, somehat negative, neutral, somewhat positive, very positive). By keeping the survey brief and lightweight, we were able to achieve strong compliance, with users answering the survey on over 75% of the occasions in which it was shown. Time of day was measured based on the UTC timestamp recorded in our database adjusted for the user's reported time zone, and was thus available for every gameplay. Users also provide basic demographic information including age, gender, and level of education as part of the standard Lumosity training experience, and, in some cases, self-perceived chronotype. In this poster, we explore the

relationships between age, gender, immediate sleep and mood and time of day in this large dataset using mixed effects models that control for individual differences between users, allowing us to measure intra-individual effects of these variables and the ways in which they interact.

Disclosures: **D.A. Sternberg:** A. Employment/Salary (full or part-time):; Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **K. Katovich:** A. Employment/Salary (full or part-time):; Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **J.L. Hardy:** A. Employment/Salary (full or part-time):; Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **M. Scanlon:** A. Employment/Salary (full or part-time):; Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs.

Poster

744. Attention: Individual Differences

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 744.18/SS23

Topic: F.01. Human Cognition and Behavior

Support: UL1-DE019580

RL1DA024853

T32DA024635

Title: Relationships of D1- and D2-type striatal dopamine receptor availability and motor response inhibition

Authors: ***C. L. ROBERTSON**[1,5], K. ISHIBASHI[2,5], A. K. BROWN[2,5], D. G. GHAHREMANI[2], E. CONGDON[2], F. SAAB[2], T. CANNON[6], M. A. MANDELKERN[5,7], R. M. BILDER[2,3], E. D. LONDON[1,2,5,4];
[1]Mol. and Med. Pharmacol., [2]Psychiatry and Biobehavioral Sci., [3]Psychology, [4]Brain Res. Inst., UCLA, Los Angeles, CA; [5]Veterans Admin. Greater Los Angeles Healthcare Syst., Los Angeles, CA; [6]Psychology and Psychiatry, Yale, New Haven, CT; [7]Physics, UCI, Irvine, CA

Abstract: Striatal dopaminergic neurotransmission is essential to the neural circuitry that mediates motor response-inhibition. Recent reports from an animal study (1) corroborate a model in which D1-and D2-type receptors promote competing processes via modulation of the direct (go) and indirect (no-go) pathways, respectively (2). Despite known associations of D2-type receptors with response inhibition (3), the relationship between D1-type receptors and response inhibition has not been tested in humans. We hypothesized that individual differences in both striatal D1- and D2-type receptors would be related to measurements of capacity for response inhibition. To test this, we examined relationships between performance on two well-established tests of motor response-inhibition, the Stop-Signal Task and a Go/No-go Task, and measurements of D1- and D2-type receptor availability using the PET radioligands ^{11}C-NNC-112 and ^{18}F-fallypride, respectively. Thirty-one healthy volunteers (15 men, mean age = 30.68, SD = 8.3) completed response inhibition tasks and MRI scans. Twenty-six subjects underwent ^{11}C-NNC-112 PET scans to assay D1-type receptor binding potential (BP_{ND}) and twenty-seven underwent ^{18}F-fallypride PET scans to determine D2-type BP_{ND}. Response-inhibition capacity was indexed by stop-signal reaction time (SSRT) in the Stop-Signal Task and commission errors on the Go/No-go Task. Partial correlations were used (controlling for sex and age) to test the relationship of striatal BP_{ND} using each tracer to response-inhibition capacity indices for each task. Striatal D2-type BP_{ND} showed a significant correlation with SSRT (r=-0.478, p=0.021), post-hoc tests reveal a strong relationship in the dorsal striatum (r=-0.544, p=0.007). Notably, striatal D1-type BP_{ND} was also significantly negatively correlated with SSRT (r=-0.624, p = 0.003), and post-hoc tests revealed a significant relationship in the dorsal striatum (r=-0.548, p=0.012). There were no significant correlations observed with response inhibition capacity on the Go/No-go Task. The results suggest both striatal D1- and D2-type receptors are important to the neurocircuitry of response inhibition, possibly contributing in different ways. The data also identify the dorsal striatum as a locus of dopaminergic control over response inhibition capacity in the Stop-Signal Task. These results suggest that different neurochemical mechanisms may subserve response-inhibition capacity assessed in the Stop-Signal Task, compared to that assessed in the Go/No-go Task. 1 Eagle, J Neurosci, 2011. 2 Logan & Cowan, J Exp Psychol Hum Percept Perform, 1984. 3 Ghahremani, J Neurosci, 2012.

Disclosures: C.L. Robertson: None. **K. Ishibashi:** None. **A.K. Brown:** None. **D.G. Ghahremani:** None. **E. Congdon:** None. **F. Saab:** None. **T. Cannon:** None. **M.A. Mandelkern:** None. **R.M. Bilder:** None. **E.D. London:** None.

Poster

744. Attention: Individual Differences

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 744.19/SS24

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant

Title: Moment to moment BOLD signal variability in resting state networks correlates with cognitive performance in a traumatic brain injury sample

Authors: *A. RIGON, M. C. DUFF, M. W. VOSS;
Univ. of Iowa, Iowa City, IA

Abstract: Within person moment-to-moment brain signal variability has been shown to be a predictor of cognitive performance in both healthy subjects and clinical populations. Using a statistical method of mean squared successive difference (MSSD) (Samanez-Larkin et al, 2010), we investigated how resting state BOLD signal variability correlates with performance on Wechsler Adult Intelligence Scale (WAIS) subtests in a traumatic brain injury (TBI) sample (N=15) and in a matched normal comparison group. We collected 6 minutes of resting state fMRI data and performed standard processing with a custom toolbox, including ICA-based denoising. We then examined neural variability in the Default Mode Network (DMN) and the Frontal Executive Network (FEN); networks were defined with publicly available maps from a meta-analysis by Smith et al (2009). Preliminary analysis revealed marginally significant lower neural variability in the right FEN for the TBI sample. In addition, in the TBI group higher neural signal variability in the right FEN positively correlated with the Processing Speed index ($r=.57$, $p<0.05$), with the Verbal Comprehension index ($r=.58$, $p<0.05$) and with the Visual Puzzle subscore of the Perceptual Reasoning index ($r=.60$, $p<0.05$). Interestingly, further analysis revealed significantly higher neural variability in the DMN for the TBI group when compared with NC (p-val HERE?); in the TBI sample, neural variability in the DMN negatively correlated with the Symbol Search ($r=-.73$, $p<0.01$) and Visual Puzzle ($r=-.54$, $p=0.05$) subtests and with the Perceptual Reasoning index ($r=-.54$, $p=0.05$). Our initial results indicate the existence of an optimal brain signal variability interval, which could be differently disrupted in separate resting state networks following TBI. Future directions include further increasing the sample size and examining the relationship between neural variability and other cognitive indexescomparing the relative gain in predicting cognitive performance from variability of signal compared to other signal measures such as amplitude and functional connectivity.

Disclosures: A. Rigon: None. **M.C. Duff:** None. **M.W. Voss:** None.

Poster

744. Attention: Individual Differences

Location: Halls A-C

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Program#/Poster#: 744.20/SS25

Topic: A.10. Adolescent Development

Support: NIH Grant# R01HD061414

Title: Distinct patterns of cortical regionalization linked to individual differences in phonological and inhibitory functions in children

Authors: *L. B. CURLEY[1], E. NEWMAN[2], T. BROWN[3], K. S. MADSEN[5], M. R. GONZALEZ[1], A. M. DALE[4], T. L. JERNIGAN[1];
[1]Cognitive Sci., [2]Ctr. for Human Develop., [3]Multimodal Imaging Lab., [4]Radiology, UCSD, La Jolla, CA; [5]Danish Res. Ctr. for Magnetic Resonance, Copenhagen Univ. Hosp., Hvidovre, Denmark

Abstract: Many important cognitive skills emerge over the course of development and individual children vary in the rate of improvement of their skills in different domains. Here, we contrast the regional patterns of cortical surface area associated with two distinct cognitive skills: phonological processing and motor response inhibition. We hypothesized that stronger phonological processing skills would predict areal expansion of temporal lobe structures and stronger performance on an inhibitory task would predict areal expansion in the opercular region of the inferior frontal cortex. Independent of age, better phonological processing ability was associated with relatively greater cortical surface area in the posterolateral temporal lobe. In contrast, better inhibitory function was associated with relatively greater cortical surface area in the inferior frontal gyrus. These findings suggest that there may be distinct cortical surface phenotypes in the developing brain that differentiate those individuals who perform well on a phonological task from those who perform well on a motor inhibition task.

Disclosures: L.B. Curley: None. **E. Newman:** None. **T. Brown:** None. **K.S. Madsen:** None. **M.R. Gonzalez:** None. **A.M. Dale:** None. **T.L. Jernigan:** None.

Poster

745. Executive Function: Models of Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 745.01/SS26

Topic: F.02. Animal Cognition and Behavior

Support: Research grant from the Stanley Medical Institutes

Title: Determining the role of Toxoplasma derived dopamine in animal behavior

Authors: *R. MCFARLAND[1], M. V. PLETNIKOV[2];
[1]molecular microbiology and immunology, [2]Behavioral Neurobio., Johns Hopkins Univ., Baltimore, MD

Abstract: Background: The parasite Toxoplasma gondii(Toxo) is a protozoan parasite of both humans and rodents. Acute infection is associated with fetal encephalitis and brain death, while adult infections form latent intra-neuronal cysts containing tens to thousands of individual parasites. The presence of these latent cysts in humans has been shown to correlate with several psychiatric diseases, while the same latent parasitic infection in rodents has demonstrable effects on the cognitive function and fear response of those animals. Specifically latent infection with Toxo appears to induce a change in the way in which mice and rats perceive cat odors. Deficits in several important cognitive and anxiety tests have also been seen following infection with the parasite. Recent evidence has identified a tyrosine hydroxylase(TH) gene in the Toxo genome which appears be a mechanism by which the parasite produces dopamine while in the intra-neuronal cysts. The parasite has specifically been observed increasing metabolism of dopamine following encystment in mammalian neurons. The role of the TH gene and the dopamine produced by Toxo is of immediate interest to behavioral neuroscience. Methods: In collaboration with another lab, our group has received several strains of parasites. One has a disruption in the TH gene that effectively blocks expression of this enzyme. The other two are an unmodified version of the same parent Pru strain parasites and a strain with an unspecific insertion of the same disruptive element. The result is that by infecting animals with these three strains of parasites, and allowing a latent infection to develop, we can isolate the effects of parasitic tyrosine hydroxylase on behavioral changes following infection. However, this data is dependent on the three strains showing equal virulence, numbers of cysts produced in the mouse brains, as well as equal immune reaction. Results: Currently, we are blinded as to which strain is deficient in the TH pathway, but should have concluded experiments and broken the blind by November of this year. So far we have measured weight loss as an indicator of infection in 89 BALB/c mice infected with these various strains as well as control injected individuals. This profile shows an even amount of weight loss and sickness behavior in all strains relative to control. Similarly, an analysis of Toxo specific antibody generation during active and latent infection shows no difference. Counts of established cysts in 38 of those animals terminated earlier, also show us that that the disruption of TH does not impact virulence in a host. Behavioral assays are ongoing and the data should be complete and analyzed by November of this year.

Disclosures: R. McFarland: None. **M.V. Pletnikov:** None.

Poster

745. Executive Function: Models of Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 745.02/SS27

Topic: F.02. Animal Cognition and Behavior

Support: DBT-RA

Title: The immediate and delayed effects of acute stress on social behavior in rats

Authors: *K. SAXENA, P. CHAKRABORTY, S. CHATTARJI;
Natl. Ctr. for Biol. Sci., BANGALORE, India

Abstract: Severe and persistent emotional symptoms are a hallmark of stress-induced psychiatric disorders. Animal models of fear, anxiety and depression have been used extensively to capture various aspects of these behavioral problems in rodents. Accumulating evidence from animal models shows that exposure to stress elicits anxiogenic effects in rodents. However, the effects of stress-induced anxiety on social behavior remains relatively less studied. Moreover, the short-term effects of repeated stress, soon after the end of stress, were measured in a majority of earlier studies. Hence, in the present study, we examined if social interaction behaviour in male Spargue-Dawley rats (50-55 day old) is affected even 10 days after a single 2-hour session of immobilization stress. One day after acute stress, stressed animals exhibited a 33% reduction in social interaction compared to controls (Control: 271±11 s, Stress: 184±7 s). This decrease persisted up to 10 days after stress (Control: 271±11 s, Stress: 188±13 s). The number of anogenital sniffing events were also lower by 30% (Control: 27.9±2, Stress:19.7±0.9) one day after stress. This decrease in anogenital sniffing became more prominent 10 days later (43.6%). In contrast, self-grooming, a measure of individualistic behavior, was affected only 10 days later – the total time spent in self-grooming almost doubled (Control: 41.2 ±6.7 s, Stress: 80.7±14.8 s). Similarly, time spent in rearing was reduced by 48% 10 days after acute stress (Control: 30.7± 4.3 s, Stress: 15.9 ±1.8 s). Together, our results show that the impact of a single episode of stress on social behavior is manifested in distinct patterns. Social interaction between two stressed animals is affected 1 day after stress and this effect persists even 10 days later. In contrast, metrics of anxiety based on more individualistic behavior that do not involve interactions with another animal, are affected with a delay and take time to build up. This is consistent with earlier reports on the delayed anxiogenic effects of acute stress measured in the elevated plus-maze and open field tests. Thus, social behavior may be more sensitive to the detrimental effects of stress both in terms of its immediate impact and its persistence even after the termination of stress.

Disclosures: **K. Saxena:** None. **P. Chakraborty:** None. **S. Chattarji:** None.

Poster

745. Executive Function: Models of Disorders II

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 745.03/SS28

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant MH100652

NIH grant MH072672

NARSAD Young Investigator grant 44096

Title: Ketamine corrects reversal learning deficits induced by chronic cold stress -possible involvement of STAT3

Authors: ***M. GIROTTI**, M. S. PATTON, D. A. MORILAK;
Pharmacol., Univ. Texas Hlth. Sci. Ctr., SAN ANTONIO, TX

Abstract: We have previously found that ketamine, a NMDA receptor antagonist shown to revert behavioral and cellular consequences of stress, reduces the effects of chronic stress in a rodent model of coping style preference, the defensive burying test (DB). Specifically, we found that chronic stress induced a shift from active to passive coping behavior, a less adaptive means to respond to an unexpected stress, while ketamine (10 mg/kg i.p.) administered 24 h before testing restored active coping behavior in stressed animals. In addition, our laboratory has shown that cognitive set-shifting ability - a form of cognitive flexibility mediated predominantly by the medial prefrontal cortex - is impaired by chronic unpredictable stress in rats. Notably, acute ketamine administration 24 h before testing also restored optimal performance in the set-shifting test in animals subjected to chronic stress. In this study we investigated the effects of ketamine on another type of cognitive flexibility, reversal learning. Reversal learning is mainly mediated by orbitofrontal cortex (OFC) and is impaired by chronic intermittent cold (CIC) stress in rats. We found that ketamine (10 mg/kg, i.p.), given 24 h prior to behavioral testing restored reversal learning ability in CIC-stressed rats. We also observed that ketamine increased the levels of nuclear phosphorylated STAT3 in the OFC. Thus, we are currently investigating the hypothesis that increases in STAT3 signaling may be a mediator of the positive effects of ketamine on reversal learning.

Disclosures: **M. Girotti:** None. **M.S. Patton:** None. **D.A. Morilak:** None.

Poster

745. Executive Function: Models of Disorders II

Location: Halls A-C

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Program#/Poster#: 745.04/SS29

Topic: F.02. Animal Cognition and Behavior

Support: NIMH053851

NIMH072672

Title: Prefrontal cortical plasticity and behavioral effects of chronic stress

Authors: ***J. D. JETT**, D. A. MORILAK;
Pharmacol, Univ. Texas Hlth. Sci. Ctr, SA, San Antonio, TX

Abstract: Deficits in cognitive flexibility have been associated with the onset and maintenance of stress-related neuropsychiatric illnesses, such as depression and anxiety disorders. Cognitive flexibility, the ability to modify behaviors in response to changes in the environment, is mediated by medial prefrontal cortex (mPFC) function. Previously, we showed that chronic unpredictable stress (CUS) induced impairments of cognitive flexibility specific to mPFC function in rats, as measured by the Attentional Set-Shifting Test (AST). Additionally, administering adrenergic antagonists locally into the mPFC during CUS prevented the stress-induced deficits in cognitive flexibility. These results suggested that repeated stress-evoked NE activity during CUS compromises mPFC function. Subsequently, we found that an acute, low-dose, systemic administration of the NMDA receptor antagonist, ketamine, administered 24 hrs prior to testing, reversed the CUS-induced cognitive impairments on the AST. Further, this antidepressant-like effect of ketamine was associated with a drug-induced LTP-like facilitation of mPFC response to a single pulse stimulation of the ventral hippocampus (vHipp). Moreover, induction of LTP in the mPFC by high frequency stimulation in the vHipp recapitulated the therapeutic effects of ketamine on the forced swim test, a behavioral measure of antidepressant-like efficacy. Thus, we hypothesized that compromised plasticity in the mPFC may account for other behavioral deficits induced by CUS, and that effective therapeutic interventions may restore or protect LTP-like plasticity in the mPFC. In our first experiment, rats exposed to CUS showed an increase in immobility on the shock probe defensive burying (SPDB) test, a measure of active and passive coping behavior. However, by contrast with previous results on the forced swim test, induction

of LTP in the mPFC by high frequency stimulation in the vHipp did not restore active coping in CUS treated rats ($p = 0.8$). This evidence suggests that neuronal circuits other than the vHipp-mPFC pathway underlie behavioral effects on the SPDB test. The next experiment is ongoing to determine if CUS-induced cognitive deficits on the AST are associated with changes in LTP or LTD in the mPFC, and to test whether adrenergic antagonist treatment during CUS prevents such changes in neuronal plasticity.

Disclosures: J.D. Jett: None. **D.A. Morilak:** None.

Poster

745. Executive Function: Models of Disorders II

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: Science Foundation Ireland (SFI), Short Term Travel Fellowship

Title: As time goes by: The gradual impact of chronic stress on rodent hippocampal structure and function

Authors: *M. RAHMAN[1], C. KERSKENS[2], C. K. CALLAGHAN[2], S. CHATTARJI[1], S. M. O'MARA[2];
[1]Natl. Ctr. for Biol. Sci., Bangalore, India; [2]Trinity Col. Inst. of Neuroscience, Trinity Col. Dublin, Dublin, Ireland

Abstract: Accumulating evidence from neuroimaging and clinical studies show that exposure to prolonged and severe stress has detrimental effects on hippocampal structure and function. Animal models of chronic stress have been used to probe the underlying causes of stress-induced damage at multiple levels of neural. A majority of these studies, however, relied primarily on postmortem analysis at the end of repeated stress. Relatively little is known about gradual changes in hippocampal structure, and its behavioral consequences, during the course of repeated stress in the same animal. Here, we examined how chronic immobilization stress (2 h/day for 10 days) affected hippocampal volume and hippocampal-dependent spatial learning in adult male Wistar rats. Specifically, we analyzed if and how changes in hippocampal volume predict impairment of spatial memory *in the same animal* during and after the 10-day stress protocol. Using a 7T MRI scanner, we quantified a gradual decrease in hippocampal volume over the course of the 10-day chronic stress. A significant decrease in volume was seen as early as the 3rd

day of stress. Further, loss of left hippocampal volume was greater that the right hippocampus. The same rats, when tested on the Morris Water Maze task during days 4, 5, & 6 exhibited only a mild deficit in the retention, but not acquisition, of spatial memory for the hidden platform. However, after the end of chronic stress, the same animals showed a significant deficit in the Object Displacement Task. These behavioral results point to a gradual build-up in spatial memory deficits over the course of chronic stress. Consistent with earlier human studies, the reduction in hippocampal volume was strongly correlated with the spatial memory deficit seen at the end of chronic stress. Strikingly, the decrease in hippocampal volume even on day 3 was correlated with the eventual spatial memory deficit seen after the end of stress. This suggests that animals that were worst affected both in terms of memory deficits and hippocampal atrophy at the end of chronic stress, show relatively early signs of stress-induced impairment and continue on a trajectory of steady decline as the stress is repeated. Taken together, these findings suggest that not only is loss of hippocampal volume linked to memory deficits caused by repeated stress, but it may also serve as an early indicator for the eventual cognitive impairment seen in stress-related psychiatric disorders.

Disclosures: M. Rahman: None. **C. Kerskens:** None. **C.K. Callaghan:** None. **S. Chattarji:** None. **S.M. O'Mara:** None.

Poster

745. Executive Function: Models of Disorders II

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Program#/Poster#: 745.06/SS31

Topic: F.02. Animal Cognition and Behavior

Title: Ketamine provides anti-depressant efficacy in treatment-resistant rats following inflammation

Authors: J. B. PRICE[1], *S. J. TYE[2];
[2]Psychiatry & Psychology, [1]Mayo Clin., Rochester, MN

Abstract: Recent literature regarding the application of ketamine in depression research has flourished due to the drug's unique and rapid mechanism of antidepressant activity. Ketamine is known to work through the glutamatergic system and elicit a rapid antidepressant response, starkly contrasting both the mechanisms of action and response latencies inherent in traditional pharmacological antidepressants. Despite the growing trend of research characterizing the metabolic pathways influenced by ketamine, there is a relative dearth of research investigating

ketamine's role in treatment-resistant depression (TRD) within the context of the inflammatory response commonly attributed to a depressive state. The purpose of this study is to provide a rodent model of TRD for future research in this area. This study demonstrates that ketamine (10mg/kg) reduces immobility during the forced swim test by rats previously injected with lipopolysaccharides (LPS ; 750-1250 ug/kg) ($p < .001$; $n = 5$) over a period of 5 days. This reduction in immobility provided by ketamine was also found in animals modeling TRD via daily administration of adrenocorticotrophic hormone (ACTH ; 100ug/day) ($p < .05$; $n = 6$). Validity of these results was corroborated by the open field test, which indicated that observations of immobility while swimming were not due to pre-existing motor deficit. Sucrose preference data further indicated that rats administered ACTH increased in sucrose preference following administration of LPS, suggesting a possible compensatory mechanism of sucrose consumption during the inflammatory response. Animals receiving saline in place of LPS did not display an increase in sucrose consumption. Together, these results demonstrate the efficacy of ketamine in mitigating the depressive-like behaviors elicited by the inflammatory response in an animal model of TRD.

Disclosures: J.B. Price: None. **S.J. Tye:** None.

Poster

745. Executive Function: Models of Disorders II

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Program#/Poster#: 745.07/SS32

Topic: F.02. Animal Cognition and Behavior

Support: RO1 MH069860

RO1 MH049698

T32DK059803

Title: 'Sequester' stress: A novel animal model for depression-like behavior

Authors: *B. L. KOPP[1], B. MYERS[2], M. SOLOMON[3], J. P. HERMAN[2];
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Abstract: Over twenty percent of people worldwide suffer from depression. Currently, there are numerous animal models that mimic features of depression. Most models use exogenous

application of physical and/or psychological stressors. However, life stress in humans is often associated with removal of positive stimuli. To model loss of reward in rats, we developed a new paradigm that introduces loss of positive stimuli as a stressor. We exposed male Sprague-Dawley rats to 30 days of environmental enrichment, consisting of 10 rats per 1m3 cage, with various toys rotated every 4-5 days. After the 30 days, they were removed and placed into isolation in standard housing, thereby constituting an 'enrichment sequestered' (ES) condition. Over a period of 6 days, we analyzed several behaviors relevant to mood symptoms, including novel object interaction in an open field, social interaction, and forced swimming behavior. We compared three groups: ES, continuous environmental enrichment (EE), and control (CON, standard single housing). In the open field, both ER and EE rats spent more time in the center investigating the novel object, possibly a consequence of frequent exposure to novel objects as part of the EE regimen. The ER group did not differ from controls in social interaction. However, in the forced swim test ER rats had increased immobility relative to both EE and CON, consistent with increased depression-like behavior. Furthermore, EE rats had decreased forced swimming-induced cFos immunolabeling in the basolateral amygdala and rostral infralimbic (IL) division of the prefrontal cortex. In contrast, decreased Fos activation was confined to the IL in ES rats, suggesting differential stress engagement of this intimately interconnected structures as a result of resource loss (removal of enrichment). Overall, our results suggest that enrichment retraction elicits depression-like behavior, in the absence of concomitant anxiety-like behavior. These results suggest that we can model mood change associated with loss of resources in rodents, and that such changes may result from altered activity of the basolateral amygdala.

Disclosures: B.L. Kopp: None. **B. Myers:** None. **M. Solomon:** None. **J.P. Herman:** None.

Poster

745. Executive Function: Models of Disorders II

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Topic: F.02. Animal Cognition and Behavior

Support: PAPIIT IN305012-3

CONACYT 167016

CONACYT 286897

Title: Prospective timing behavior in spontaneously hypertensive rats

Authors: *V. ORDUÑA, E. TRUJANO;
UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, MEXICO, Mexico

Abstract: The behavior of spontaneously hypertensive rats (SHR) has been extensively studied in order to evaluate its similitude with the behavior of patients with Attention Deficit Hyperactivity Disorder (ADHD). Because ADHD patients have deficits in time perception, the processing of time has been extensively studied in SHR; it has been reported, using retrospective and immediate timing tasks, that timing is not altered in this strain. The goal of the present experiment was to analyze the performance of SHR, Wistar Kyoto, and Wistar rats in a prospective timing task: the time-left procedure. In this procedure, organisms have to choose between an option C where reinforcement is available after a fixed delay (v.g. 60 s), and another option S where reinforcement is available after a shorter fixed delay (v.g. 30 s). Time at the comparison option C starts to run since trial begins, whereas time at the standard option S starts to run T seconds after trial begins (the choice moment, the independent variable). When T is larger than 30 s, reinforcement is closer for option C, while when T is shorter than 30 s, reinforcement is closer in option S. The experimental question is how an organism distributes its behavior between both options as a function of T. In our experiment, T was varied from 0 to 55 s in multiples of 5. Preference for C was described by a logistic function. The parameters of this function reflect some characteristics of the timing behavior: The indifference point reflects the accuracy, while the variability around the mean reflects the precision. We found that the parameters of SHR were not different to those of Wistar rats, a result that suggests that prospective timing is not altered in SHR.

Disclosures: V. Orduña: None. **E. Trujano:** None.

Poster

745. Executive Function: Models of Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 745.09/SS34

Topic: F.02. Animal Cognition and Behavior

Support: NSERC Discovery grant

Title: Executive functional changes in a putative neurodevelopmental model of schizophrenia made by neonatal lesions of the prefrontal cortical subplate

Authors: S. J. DESAI, F. JUNG, Q. YAO, B. L. ALLMAN, *R. RAJAKUMAR;
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Abstract: Partial ablation of subplate neurons of the developing prefrontal cortex (PFC) in neonatal rat pups results in the adult emergence of positive and negative symptom-like features and structural abnormalities that are consistent with schizophrenia. Within the PFC, these changes include: 1) loss of volume; 2) altered laminar distribution of parvalbumin immunoreactive neurons and terminals, as well as thalamocortical and dopamine fibers; 3) decreased synaptophysin immunolabeling, and; 4) loss of GABA transporter-1 immunoreactivity restricted to upper lamina of the PFC. Based on these neurochemical and synaptic abnormalities in the dopaminergic, GABAergic and glutamatergic systems within the PFC, we hypothesize that subplate lesioned rats will show behavioural deficits in tasks involving cognitive flexibility, working memory and reasoning (i.e., 'executive functions'). Neonatally-lesioned and sham-operated (control) groups of animals at 14-20 weeks of age will undergo an operant conditional paradigm-based attentional set-shifting task, novel object recognition task, and a 5-choice serial reaction-time task. Our preliminary results in the attentional set-shifting task revealed that the average number of trials required by the lesioned group to achieve the performance criterion was greater than that of the control group, but this difference was not statistically significant. Additional testing and analyses are ongoing. Collectively, our findings will characterize whether the neonatal disruption of the subplate in the PFC impairs executive functioning in this putative neurodevelopmental animal model of schizophrenia.

Disclosures: S.J. Desai: None. **R. Rajakumar:** None. **F. Jung:** None. **Q. Yao:** None. **B.L. Allman:** None.

Poster

745. Executive Function: Models of Disorders II

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Topic: F.02. Animal Cognition and Behavior

Support: MH087978

MH091372

Title: Transcriptional changes in the prefrontal cortex linking different forms of gestational adversity to specific deficits of executive function

Authors: *N. M. GRISSOM, C. HERDT, J. DESILETS, J. LIDSKY-EVERSON, T. M. REYES;
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Abstract: Deficits in executive function, including poor attention or impulse control, characterize a number of mental disorders, including schizophrenia, autism, and attention deficit/hyperactivity disorder (ADHD). Executive function is mediated by the medial prefrontal cortex (PFC), which displays markers of epigenetic dysregulation in these disorders. Adverse early life experiences, such as poor nutrition during gestation, alter gene expression and DNA methylation in PFC neurons. However, it is unknown how alterations in PFC transcription relate to specific executive function deficits. Behavioral deficits in executive function disorders can occur independently, for example impulsivity in the absence of inattention. Currently, neither specific etiologies nor molecular deficits have been linked to dissociable executive function domains. Here, we demonstrate dissociable deficits in executive function in male and female mice, solely due to poor maternal nutrition via high-fat or low-protein diets. In the 5-choice serial reaction time task (5-CSRTT), gestational exposure to a high-fat diet promoted impulsivity, while exposure to a low-protein diet led to marked inattention. These animals also displayed dissociable deficits in motivated behavior assessed by Pavlovian conditioned approach and progressive ratio. Following this extensive behavioral characterization, we assayed PFC gene expression using a targeted PCR array and found that both maternal diets significantly increased overall transcription in PFC. Unsupervised cluster analysis of the relationships between individual transcripts and behavioral outcomes revealed a cluster of primarily epigenetic modulators whose overexpression was linked to executive function deficits. The overexpression of four genes, DNA methyltransferase 1 (DNMT1), delta opioid receptor (OPRD1), cannabinoid receptor 1 (CNR1), and catechol-o-methyltransferase (COMT), was strongly associated with overall poor performance. All 5-CSRTT deficits were associated with DNMT1 upregulation, while impulsive behavior could be dissociated from inattention by overexpression of distinct epigenetic regulators as well as OPRD1 or COMT, respectively. These data provide molecular support for dissociable domains of executive function.

Disclosures: **N.M. Grissom:** None. **C. Herdt:** None. **J. Desilets:** None. **J. Lidsky-Everson:** None. **T.M. Reyes:** None.

Poster

745. Executive Function: Models of Disorders II

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Program#/Poster#: 745.11/SS36

Topic: F.02. Animal Cognition and Behavior

Support: R03AA022480

Title: The effects of fetal alcohol exposure on attentional set shifting and susceptibility to distraction

Authors: *J. A. MCGAUGHY[1], D. J. CREER[1], J. A. MCBURNIE[1], P. ROBINSON-DRUMMER[2], M. E. STANTON[2];
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Abstract: Fetal alcohol spectrum disorder (FASD) is a leading cause of intellectual and developmental disabilities. This exposure negatively impacts the development of cerebellum, hippocampus, striatum and frontal cortices. Moreover, FASD causes deficits in many aspects of cognition including associative learning, working memory and attentional set shifting. These findings in humans have stimulated translational research using rats to better understand the relationship between brain changes and impairments in cognition. The present study used neonatal alcohol exposure (P4-9, 5.25 mg/kg/day), a rat model of FASD (Schrieber et al, 2013), to determine the impact of this treatment on attentional set shifting and susceptibility to distraction during adulthood. We hypothesized that FASD subjects would be impaired in tests of inhibitory control and attentional set shifting. Additionally, we hypothesized that this exposure would produce a greater susceptibility to distraction in a test of sustained attention. We used an intradimensional/extradimensional set-shifting task to assess the ability of these rats to form and shift attentional set. Interspersed among testing stages were tests of reversal learning that measured inhibitory control. Contrary to our predictions, alcohol produced a slight facilitation of performance at all stages of testing though there was no significant difference between groups on any one stage. This difference in performance may reflect increased cognitive flexibility. These same subjects were then trained in a test of sustained attention with a visual distractor (dSAT). FASD rats were more susceptible to distraction than control subjects. Previous work has shown that our alcohol exposure protocol produces morphological changes to the prefrontal cortex in rats. Subsequent to the completion of behavioral testing, we conducted post-mortem analyses of noradrenergic and cholinergic innervation of prefrontal cortices. The focus on these neuromodulatory systems is based on previous work showing that these systems contribute to attentional control. The impact of alcohol on anterior cingulate, orbitofrontal, infralimbic and prelimbic cortices was assessed. Together these data support the hypothesis that a rodent model of FASD produces changes in executive function. Though previous work in humans has shown FASD is associated with problems in set-shifting, the most robust demonstration of these impairments were found in children. As a result, future studies will be aimed at assessing attentional set-shifting at earlier stages of development.

Disclosures: **J.A. McGaughy:** A. Employment/Salary (full or part-time):; University of New Hampshire. **D.J. Creer:** None. **J.A. McBurnie:** None. **P. Robinson-Drummer:** None. **M.E. Stanton:** None.

Poster

745. Executive Function: Models of Disorders II

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Topic: F.02. Animal Cognition and Behavior

Support: MRC Programme Grant to ACR

MRC Career development award to HFC

Title: Fronto-subcortical circuits and avoidance behaviour: Differential contributions of the orbitofrontal and ventrolateral PFC

Authors: ***H. F. CLARKE**[1,2], N. HORST[3], A. C. ROBERTS[1,2];
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Abstract: Elevated fear and anxiety are key symptoms of affective disorders, and are associated with dysfunction in fronto-subcortical circuits that include the amygdala and anterior hippocampus (antHipp). Interestingly, despite extensive segregation of executive behaviours at the level of the prefrontal cortex, the differential role of the ventrolateral prefrontal (vlPFC; Brodmann's area 45/12) and orbitofrontal (OFC; Brodmann's area 11) cortices in anxiety behaviours is uncertain. Indeed lesions of both areas in the marmoset result in increased anxiety and conditioned fear (Agustín-Pavón C et al., 2012, Biol Psychiatry, 72(4):266-72), and both areas are activated in human anxiety (Campbel-Sils et al., 2011, Neuroimage, 54, 689-696). However, it is not known if they also modulate the impact of anxiety on choice behaviour. The present study therefore investigates the role of the vlPFC and the OFC in the control of instrumental responding by unconditioned mildly aversive stimuli. Marmosets were trained to respond to 2 identical stimuli presented on either side of a touch sensitive computer screen that were on independent, variable-interval reward (5 sec banana milkshake) schedules, occasionally overlaid with variable punishment (0.3 sec 117dB loud noise) on one side only. They were then implanted with intracerebral cannulae targeting either the vlPFC alone or the OFC, combined with the amygdala and antHipp to allow temporary inactivation (0.1mM

muscimol/1.0mMbaclofen; 0.5µl infused at 0.25µl/min) of these regions. Compared to saline, vlPFC inactivation caused animals to bias their responding away from the punished side on the infusion day. This effect was blocked by valium (0.25mg/kg 30min pretreat), suggesting an anxiety component to the anti-punishment bias. In contrast, OFC inactivation had no effect on the infusion day but resulted in a profound bias away from the punished side the next day (when neither inactivation or punishment occurred). This effect was blocked by both valium and inactivation of either the amygdala, antHipp, or a disconnection of the two, on that next day. These results demonstrate the dissociable roles of the vlPFC and the OFC in the regulation of responding to unconditioned punishment. We hypothesise that the vlPFC is important for reconciling conflict between punishment and reward in the context of response selection, with its absence allowing punishment to dominate. In contrast, inactivation of the OFC has no impact on response selection, per se, but results in enhanced punishment memory within an amygdala-antHipp circuit, and therefore suggests that the OFC directly modulates amygdala plasticity.

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Poster

745. Executive Function: Models of Disorders II

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Programme Grant (no. 089589/Z/09/Z)

Title: Neural correlates of quinpirole-induced checking behavior on the observing response task for rats

Authors: *L.-S. D'ANGELO[1,2], B. JUPP[1,2,3], D. M. EAGLE[1,2], T. W. ROBBINS[1,2];
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Abstract: Excessive checking is a common, debilitating symptom of obsessive-compulsive disorder (OCD). In an established model of OCD, rats treated chronically with the dopamine D2/3 receptor agonist quinpirole show compulsive checking-like behavior (e.g., towards objects in an open field), indicating dopaminergic modulation of checking-like behavior. We recently demonstrated quinpirole-induced 'checking' in an operant Observing Response Task (ORT). In

the ORT, rats pressed an 'observing' lever for information about the location of an 'active' lever that provided food reinforcement. Quinpirole (0.5 mg/kg, 10 treatments) selectively increased checking, both observing lever presses (OLP) for information but also 'excessive' (inconsequential) OLP (EOLP) while the light was on. However, the factors underlying responsivity to quinpirole, and subsequent development of excessive observing on the task, are not fully understood. Individual differences in responsivity to quinpirole have been shown across several studies, with rats showing both low and high response to quinpirole on the ORT. Little is known of the neurochemical changes associated with such individual differences or with behavioral sensitisation to quinpirole. Neurobiological research on OCD supports involvement of cortico-basal ganglia circuits, as well as dysregulation of several neurotransmitter systems, including serotonin (5-HT), dopamine (DA) and gamma-amino-butyric acid (GABA) in this disorder. Thus we investigated neural correlates of behavioral sensitisation to chronic quinpirole as well as individual differences in quinpirole-induced checking on the ORT. Using autoradiography, we quantified DA transporter (DAT), 5-HT transporter and D1, D2/D3, 5-HT2 and GABA(A) receptor binding in selected regions of the prefrontal cortex and striatum in vehicle-treated rats and quinpirole-treated rats expressing low and high response to quinpirole on the ORT. High responders to quinpirole (QH) exhibited significantly higher binding for GABA(A) receptors in the dorsal striatum and nucleus accumbens core compared with both vehicle-treated and low responder rats (QL). Both QH and QL rats exhibited significantly higher GABA(A) binding in the nucleus accumbens shell compared with vehicle-treated rats. QL rats also showed significantly lower binding for DAT in the prelimbic and infralimbic cortex compared with both QH and vehicle-treated rats. These results indicate that altered dopaminergic and GABAergic function in cortico-striatal circuits may underlie individual variation in the response to quinpirole on the ORT, with potential relevance to the etiology and treatment of OCD.

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Poster

745. Executive Function: Models of Disorders II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Campus France PHC Procope

LIA CNRS-NYU LearnEmoTime

ANR TDE

Title: Age-related alteration of decision making in the BACHD rat model for Huntington's disease

Authors: N. EL MASSIOUI[1], S. YAGUE[1], N. ADJEROUD[1], G. MANFRE[2], S. BOSSI[1], O. RIESS[2], P. ALLAIN[3], N. SAMSON[1,3], L. YU-TAEGER[2], H. NGUYEN[2], *V. DOYERE[1]; [1]CNRS-UMR 8195 CNPS, ORSAY, France; [2]Dept. of Med. Genet., Univ. of Tuebingen, Tuebingen, Germany; [3]Processus de pensées et interventions, Univ. d'Angers, Angers, France

Abstract: Huntington disease (HD) is an autosomal dominantly inherited, progressive neurodegenerative disorder. Executive dysfunctions can be observed with high prevalence before motor symptoms. To characterize the progression with age of these dysexecutive symptoms, we tested BACHD rat (a "full length" model of HD) in a rat version of the Iowa gambling task (RGT; Rivalan et al, 2009). BACHD and WT rats were trained at three different ages: 8 weeks, 7 months and 17 months to test presymptomatic and symptomatic animals. Rats were first trained for 5-7 days to nose-poke in 5-holes skinner boxes to obtain food pellets. They were then tested in the RGT for a 1-hour session during which rats had to choose between holes for advantageous (small reward but short penalty) or disadvantageous consequences (large reward but long penalty). Moreover, to assess choice vs action impulsivity at the presymptomatic age, 4-5 months old BACHD and WT rats were trained in a delay discounting task in which 2 levers were available, one associated with immediate delivery of 1 food pellet whereas the other was associated with 3 delayed food pellets. Another group of animals of the same age were trained in a differential reinforcement of low rates task in which reinforcement is contingent upon responses which are spaced t seconds (5s followed by 10s) or more from the previous response. Seventeen months old BACHD rats choose less advantageous choices than WT rats while younger BACHD animals behaved as efficiently as WT. However, at a young presymptomatic age (4 months), BACHD rats showed high impulsive action as well as high impulsive choice behavior compared to WT. In conclusion, presymptomatic animals demonstrate a high level of impulsivity which did not however alter their choice efficiency in the gambling task, although behavior in that latter task was altered in symptomatic animals. This dissociation suggests an age-related differential alteration of underlying neurobiological systems.

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Poster

745. Executive Function: Models of Disorders II

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Topic: F.02. Animal Cognition and Behavior

Support: Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (JSPS)

Title: Characterizing behavioral flexibility impairment in mouse models of cognitive deficits induced by N-ethyl-N-nitrosourea mutagenesis and p-chlorophenylalanine

Authors: ***T. ENDO**[1,2], S. BENNER[1], W. LING[1], N. ENDO[1], E. KIMURA[1], T. FURUSE[3], S. WAKANA[3], H. BITO[2], C. TOHYAMA[1], M. KAKEYAMA[1,4];
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Abstract: Behavioral flexibility is an intellectual ability that is endowed for humans to adapt to an ever-changing environment. Previously, we reported a novel assay for behavioral flexibility using mouse models (Endo et al., Behav. Brain Res., 2011; Endo et al., PLOS ONE, 2012). This assay was based on a behavioral sequencing task (i.e., reward retrieval by visiting alternately to two distantly-positioned chambers) and its serial reversals (changes of the positions of rewards), using a fully computerized testing apparatus for group-housed mice (IntelliCage). Here, we have characterized behavioral flexibility in three mouse models of cognitive deficits, i.e., mice with a missense mutation in either Grin1 (NR1) or Tuba1 gene produced by N-ethyl-N-nitrosourea (ENU) mutagenesis project, and mice with serotonin-depletion by treatment with p-chlorophenylalanine (PCPA). The Grin1 and Tuba1 mutant mice showed a significantly faster acquisition of the original learning, while they consistently exhibited an abnormal sign in behavioral flexibility, i.e., a significantly higher rate of visits to the previously rewarded corners in the following serial reversals, compared to the wild-type control. On the other hand, the PCPA-treated mice behaved normally in the phase of the original learning, but showed a

significantly higher rate of error visits in the first reversal, compared to saline-treated control mice. In addition, a number of perseverative nose pokes were significantly increased in the PCPA-treated mice, suggesting an abnormal sign in behavioral flexibility. The present results demonstrated that these mouse models of cognitive deficits develop impairments in behavioral flexibility. In conclusion, the present study proposes mouse models which are considered to mimic the psychiatric disorders with executive function deficits in humans.

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Poster

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Program#/Poster#: 745.16/SS41

Topic: F.02. Animal Cognition and Behavior

Title: Anxious phenotype plus environmental stress are related with brain DNA damage

Authors: ***D. DIMER LEFFA**[1], G. Z. RÉUS[1], F. PETRONILHO[1], H. M. ABELAIRA[3], A. S. CARLESSI[3], J. L. FERNADEZ[4], A. P. DAMIANI[3], V. M. ANDRADE[3], J. QUEVEDO[2]; [1]Psychiatry and Behavioral Sci., [2]The Univ. of Texas Hlth. Sci. Ctr. At H, Houston, TX; [3]Univ. of Southern Santa Catarina - UNESC, Criciuma, Brazil; [4]Pontifical Catholic Univ. of Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: Anxiety is described as a psychological, physiological, and behavioral state induced in animals and humans by a threat to well-being or survival, either actual or potential. In addition, depressive and anxiety disorders commonly occur together in patients. Still is a relationship between DNA damage and mood disorders. So, the present study investigated the effects of stress on behavior and brain DNA damage in rats with anxious phenotype. To this we used Wistar rats with High - and - Low Freezing (CHF, CLF), which present anxious phenotype and we submitted the CHF, CLF and control rats to chronic mild stress protocol, constituting 6 groups: 1) no anxiety + control; 2) no anxiety + stress; 3) CHF + control; 4) CHF + stress; 5) CLF + control; 6) CLF + stress. After, all animals were submitted at elevated plus maze and forced swimming tests. Moreover, genotoxic parameters were assessed in the brain areas: prefrontal cortex, hippocampus, amygdala and nucleus accumbens by comet assay. The results showed that animals with CHF stressed spent less time in arms open on elevated plus maze test,

when compared to animals with high freezing and control animals. Furthermore, the number of entries into the open arms was lower in rats with CHF stressed, compared to those not receiving stress. In the forced swim test were evaluated immobility, climbing and swimming times. At the time of immobility no significant differences between the experimental groups were shown. However, animals with CHF and stressed had an increased in swimming time and a decrease in climbing time, when compared to control stressed animals. In relation to genotoxicity in brain samples, prefrontal cortex no showed statistical difference between groups evaluated. However, hippocampus showed high levels of DNA damage in all groups when compared control group no stress. The same result was found in amygdala, with exception at control group plus stress. In nucleus accumbens the animals with CHF stressed or no stressed presented increased levels of DNA damage when compared to the other groups. In conclusion, the animals with anxious phenotype and submitted to stress protocol presented more pronounced anxious. In addition, both anxiety and stress were associated with increased on DNA damage in the hippocampus, amygdala and nucleus accumbens. These results could be associated with the biochemical modification existing in mood disorders, such as oxidative stress and inflammation. Thus, the study results indicate that when there is the presence of an anxious phenotype, and there is another stimulus, as the stress, these animals are more susceptible to other behavioral and neurochemical changes related to anxiety and depression.

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Poster

745. Executive Function: Models of Disorders II

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Topic: F.02. Animal Cognition and Behavior

Support: Drexel Ventures Innovation fund grant

NIH.NIDA R01 DA 017960

NIH.NIDA R21 MH097623

Title: Functional characterization of a novel catecholamine modulator to treat cognitive dysfunction

Authors: W. XU[1], L. KEIBEL[2], D. BERNSTEIN[2], S. SIMMS[2], R. SANCHEZ[2], A. LIN[2], A. C. K. FONTANA[3], O. V. MORTENSEN[3], R. ESPAÑA[2], B. D. WATERHOUSE[2], J. S. SHUMSKY[2], *S. KORTAGERE[1];
[1]Microbiology and Immunol., [2]Dept. of Neurobio. and Anat., [3]Dept. of Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Cognitive dysfunctions associated with neurological and psychiatric disorders present a major challenge for treatment. The role of catecholamine neurotransmitters namely dopamine (DA) and norepinephrine (NE) in promoting pro-cognitive effects and their regional selectivity in the brain has been debated. However, the lack of sub-type selective agents for the various DA receptors and their potential interactions with the noradrenergic system has hampered our efforts to delineate the precise roles of DA and NE in prefrontal cortex (PFC) mediated cognitive functions. In this study, we have designed a selective dopamine D3 receptor (D3R) agonist - SK609 that also exhibits selective NET reuptake inhibition. SK609 mostly partitions into the brain with nearly 4h of half life in the brain and 1h in the blood. To further understand the role of SK609 in modulating DA/NE levels, *in vivo* recordings of extracellular DA levels using fast scan cyclic voltammetry in the core region of the nucleus accumbens were performed. The recordings confirmed the atypical profile of SK609. Since compounds with NET inhibitor activity have shown improvements in PFC mediated cognitive effects, SK609 was tested in a sustained attention task. Similar to the psychostimulant drug methylphenidate (MPH - Ritalin®), SK609 improved rodent performance in the sustained attention task according to the well-known inverted- U dose response relationship with a peak effect at 4 mg/kg. SK609-mediated improvement in task performance was reversed by either the D2/D3 receptor antagonist raclopride or the alpha1 receptor antagonist prazosin suggesting that shared DA-NE mechanisms govern sustained attention. Further studies are ongoing to evaluate the effect of SK609 on a flexible attention task (i.e. attention set shifting) to understand the role of DA/NE mechanisms in this executive function. Lastly, to examine abuse potential, we conducted SK609 self-administration studies in rats. Preliminary results indicate that rats do not self-administer SK609. Combined these results suggest that SK609 improves sustained attention via DA and NE mechanisms and may be well-suited for treating general cognitive dysfunction with a low probability of abuse or addiction.

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Poster

745. Executive Function: Models of Disorders II

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Topic: F.02. Animal Cognition and Behavior

Support: FAPESP Grant 2013/09749-4

Title: Study of Social Defeat Stress effects on nociceptive behavior in mice

Authors: *M. F. PAGLIUSI, JR[1], I. J. M. BONET[1], C. R. SARTORI[1], C. A. PARADA[1], M. C. P. ATHIÉ[1], A. S. VIEIRA[2];
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Abstract: Despite the pain is not one of the symptoms for the depression diagnosis, epidemiological studies indicate a close association between depressive disorder and chronic pain. This is evidenced by the clinical observation that patients with depression have high prevalence of chronic pain and, at the same time, patients suffering from chronic pain are also often diagnosed with depressive disorders. Several clinical and biological characteristics are shared between pain and depression, and various neuroanatomical structures, neural circuits and neurotransmitter systems exhibit similar changes in those two conditions. In Social Defeat Stress animal model, recently developed, intruder mice are exposed to resident mice from a more aggressive and more physically robust strain, experiencing brief periods of physical aggression followed by longer periods of sensory contact. The defeated mice may display a number of physiological and behavioral depressive-like characteristics, and then they are considered susceptible; furthermore, some defeated mice may not display these characteristics, and then they are considered resilient. However, there is no data regarding the pain sensibility in such animals. Thus, this study aimed to investigate the behavioral response to nociceptive stimulation, through Von Frey and capsaicin test, in mice submitted to the Social Defeat Stress model. For this, three experimental groups were established. In the control group the intruder mice were paired with mice of the same strain; in susceptible group, intruder mice were exposed to resident mice and submitted to Social Defeat Stress and have developed depressive-like behavior; and, finally, resilient group was composed by mice that also were submitted to Social Defeat Stress but have not developed depressive-like behavior. Our data showed that susceptible mice exhibited greater pain intensity in both tests when compared to both control and resilient mice. Furthermore, we found that the resilient mice showed an intermediate nociceptive threshold compared to the other two groups (susceptible and control), presenting, therefore, resiliency also to the decreasing of the nociceptive threshold. These results suggest the existence of a common process of neuronal plasticity underlying both chronic pain and stress-induced depressive disorder. Some aspects of this neuroplasticity are currently under investigation in our laboratory. Our experimental data corroborate the comorbidity between pain and depressive disorder frequently observed in the clinic.

Disclosures: **M.F. Pagliusi:** A. Employment/Salary (full or part-time):; FAPESP (Scholarship). C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UNICAMP. **I.J.M. Bonet:** None. **C.R. Sartori:** None. **C.A. Parada:** None. **M.C.P. Athié:** None. **A.S. Vieira:** None.

Poster

746. Fear and Aversive Memories: Cortex

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Title: Coherent activity in retrosplenial cortex, hippocampus, and anterior cingulate cortex during retrieval of recent and remote memory

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Abstract: Activity in retrosplenial cortex (RSC) is often reflected in human disorders characterized by aberrant memory processing: it is one of the earliest brain regions to show metabolic decline in Alzheimer's disease, and increased activation of RSC is consistently seen during retrieval of traumatic memories in PTSD patients. Oscillatory fluctuations of local field potentials play a mechanistic role in all aspects of memory processing, however, to date most studies of such activity in rodents have dealt only with memory consolidation. RSC plays a time-independent role in the retrieval of memories for fear-evoking events; thus, we explored the patterns of RSC oscillatory activity underlying retrieval of recent and remote memories. Mice were trained to fear a context paired with footshock, and then tested for memory retrieval beginning one day (recent) or 35 days (remote) later. Using a wireless NeuroLogger system (TSE Systems), we obtained simultaneous recordings of continuous LFP activity during memory retrieval from RSC as well as hippocampus and anterior cingulate cortex (ACC), two main regions implicated in processing recent and remote memories, respectively. We measured

coherence of activity between these regions through a range of frequencies along with changes in coherence during extinction of fear responses. The mechanisms by which RSC functions as a memory retrieval hub, at the level of specific oscillatory patterns, phase coherence, and cross frequency effects, are not known. Thus, dentifying specific LFP patterns has the potential to unravel subtle mechanisms of circuit interactions associated with retrieval of recent and remote memory and enable the study of their specific molecular underpinnings.

Disclosures: K.A. Corcoran: None. **J.M. Radulovic:** None. **L.M. Kay:** None.

Poster

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Support: ERC Starting Grant 281168

Title: Prefrontal circuits of contextual fear discrimination and generalization

Authors: *R. R. ROZESKE[1], S. KHODER[1], F. CHAUDUN[1], N. KARALIS[1,2], H. WURTZ[1], C. HERRY[1];
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Abstract: Environmental cues can serve as powerful predictors of threatening events; for rodents, one behavioral response to a threatening environment is freezing. Previous studies have examined freezing behavior using environmental cues that were perfectly paired with an aversive event. However, in the natural world, the predicative value of environmental cues can be unreliable or vary over time, and the neuronal circuits required for contextual discrimination during unreliability have not been extensively investigated. Here, we used a novel fear conditioning paradigm where we systematically altered contextual cues that were present during conditioning to produce periods of fear generalization and discrimination during fear testing. To identify the neuronal circuits responsible for contextual fear generalization we performed single unit and field potential recordings coupled with optogenetic manipulations in the dorsal medial prefrontal cortex (mPFC) of behaving mice. Presentation of all contextual cues that were present during conditioning produced high levels of freezing, however sequential removal of these cues reduced freezing behavior and led to contextual discrimination. Contextual discrimination was associated with elevated firing rate of mPFC pyramidal neurons. Interestingly, light-induced

activation of channelrhodopsin-2-expressing pyramidal neurons in the mPFC reduced freezing during contextual fear generalization. In contrast, light-induced inhibition of archaerhodopsin-expressing pyramidal neurons in the mPFC produced freezing to the discriminated context. These results suggest that activation of the mPFC may be part of a circuit that supports contextual fear discrimination and generalization.

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Poster

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Support: ERC Starting grant NEUROFEAR 281168

Title: Synchronized prefrontal-amygdala 4Hz oscillations mediate fear behavior

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Abstract: Recent converging evidence suggests that the expression of conditioned fear and extinction memories relies on the coordinated activity between the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA), two structures densely and reciprocally interconnected. In particular, it has been shown (i) that persistence of fear behavior is associated with an increase in the number of pairs of mPFC and BLA neurons displaying correlated neuronal activity, and (ii) that fear behavior is associated with synchronized spiking activity between mPFC output neurons that target the BLA. However, to date, the mechanisms allowing this long-range synchronization of neuronal activity between the mPFC and BLA during fear behavior remain virtually unknown. To address this question, we used single unit and local field potential (LFP) recordings in mPFC and BLA in freely behaving mice submitted to auditory fear conditioning. The simultaneous recordings in the two structures revealed a persistent synchronized 4Hz oscillation in the mPFC-BLA circuit emerging during fear conditioning, just after the delivery of the unconditioned stimulus (US). Additionally, during freezing behavior,

mPFC oscillations drive amygdala LFPs and synchronize the spiking activity between the two structures, allowing the accurate recovery of the fear memory and expression of the conditioned fear behavior. Interestingly, pairing the CS with optogenetic induction of 4 Hz oscillations in the mPFC was sufficient to produce conditioned fear behavior. Moreover, optogenetic blockade of prefrontal 4 Hz oscillations prevented fear expression. Our data suggest that synchronized 4Hz oscillations constitute a major mechanism for the temporal coordination of the activity in the prefrontal- amygdala circuit, facilitating the encoding and expression of fear memory across the circuit. These results unravel a potent oscillatory mechanism enhancing prefrontal-amygdala coupling during fear behavior.

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Poster

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Title: Cortical localization of fear memory

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Abstract: 'Where' fear-memories are formed and stored is still not well understood. To tackle this issue, we used advanced genetic tools to first investigate whether cued and contextual fear memory acquisition-consolidation and retrieval processes involve the participation of N-methyl-D-aspartate (NMDA) receptor (NMDAR) function in the lateral amygdala (LA) and basolateral

amygdala (BLA). Using recombinant adeno-associated viruses equipped with chemically-controlled inducible genetic switches, we removed the NMDAR in LA/BLA either before or after fear memory formation. Our results demonstrate that NMDAR in the LA/BLA neurons is not required for the formation and retrieval of fear memories. Second, to silence synaptic output, we developed a virus-based genetic method called virus-delivered Inducible Silencing of Synaptic Transmission (vINSIST). When synaptic output from LA/BLA to other brain regions was blocked by silencing of synaptic transmission (ST) before memory formation, both cued and contextual fear memories failed to form, as was expected. Most remarkably, when these same synaptic connections were blocked by inducible silencing of ST after the formation of fear memories, the retrieval of these memories was unaffected. These results strongly suggest that fear memories are likely formed and distributed in brain region(s) other than the LA/BLA. We are now tracking the locus of these fear memories in the prefrontal cortical regions.

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Poster

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Title: Infralimbic cortex activity is critical for the consolidation of emotional learning

Authors: *D. C. CHOI[1], K. J. RESSLER[2];
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Abstract: The medial prefrontal cortex (mPFC) is known to regulate emotional learning and memory. More specifically, previous work has found that infralimbic cortex (IL) subregions of the mPFC are critical for fear extinction. However, less is known on the role of the IL activity in regulating the consolidation of fear extinction learning. Here we utilized both optogenetic and DREADD (Designer Receptors Exclusively Activated by Designer Drugs) approaches to investigate the whether IL activity during fear extinction training, or during the extinction consolidation period is sufficient to modulate the consolidation of fear extinction. For optogenetic approaches, mice received viral delivery of AAV-CamkIIa-ChR2-eYFP into the IL for expression of ChR2 in CamKIIa-specific pyramidal cells. Mice infected with AAV-CamkIIa-eYFP expressing just eYFP reporter served as controls underwent auditory cued fear conditioning. The following day, these mice were fear extinction-trained with optogenetic stimulationg of the IL paired to with the tone presentations. During the extinction training, activation of the IL projection neurons substantially blunted the freezing responses to the CS compared to controls. The extinction retention was also examined a day later and the mice that previously received activation of IL CamKIIa neurons showed less fear. Next, for DREADD approaches, mice received viral delivery of AAV-CaMKIIa-HA-rM3D(Gs)-IRES-mCitrine into the IL, which served to drive Gs protein coupled receptor signaling in CamKIIa-specific pyramidal cells. Mice infected with AAV-CamkIIa-eYFP expressing just eYFP reporter served as controls. The advantage of DREADDs are that they can be activated by a naturally inert ligand cyclopine-N-oxide (CNO), to drive Gs protein signaling for increased excitability (increased activity) for several hours. These mice underwent cued fear conditioning. The following day, immediately following extinction training, these mice received injections of CNO to drive Gs signaling in the IL during the extinction consolidation period. The extinction retention was also examined a day later, and the mice that previously received activation of IL CamKIIa neurons (via Gs signaling) showed less fear. These data, using two site- and cell-type selective inducible activation approaches, suggest that increasing activity of IL excitatory neurons during extinction training or during the extinction consolidation period can both lead to greater fear extinction retention. Future experiments will further investigate prefrontal cell-type specific mechanisms, and whether IL-amygdala or other circuits are involved in regulating fear extinction.

Disclosures: D.C. Choi: None. **K.J. Ressler:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH065961

Title: Noradrenergic blockade stabilizes medial prefrontal single-unit activity after footshock stress and reduces fear expression in rats

Authors: *T. F. GIUSTINO[1], P. J. FITZGERALD[2], S. MAREN[1,2];
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Abstract: We have previously shown that fear conditioning is highly resistant to extinction soon after it is acquired, a state of affairs that challenges early interventions after traumatic or stressful experiences. We hypothesize that stress-induced noradrenergic hyperarousal and consequent alterations in prefrontal function contribute to this extinction deficit. Previous work in rodents suggests the prelimbic (PL) and infralimbic (IL) subdivisions of medial prefrontal cortex (mPFC) regulate conditioned responses to footshock stress. Here we examined single-unit firing in these regions in response to aversive footshock and the modulation of these responses by systemic administration of propranolol, a non-selective beta adrenoceptor antagonist, (10 mg/kg, i.p.) in male Long-Evans rats. Single-unit activity was recorded from a 16-channel microelectrode array that targeted PL (8 wires) and IL (8 wires) in the same subjects (n= 6 propranolol-treated (PROP) rats, n=5 vehicle-treated (VEH) rats). The administration of five tone-shock trials produced immediate changes in spontaneous activity among both PL and IL neurons; roughly 75% of neurons in PL and 50% in IL exhibited increases in firing rate in vehicle-treated rats. Propranolol dampened both post-shock conditioned freezing and the magnitude of the increases in firing rate among neurons in PL and IL, as well as reducing the numbers of neurons that increased their firing rate (PL, 53%; IL, 29%). Among neurons that decreased their firing rate after shock, only IL neurons were sensitive to propranolol administration. Interestingly, neurons in PL (VEH, 25%; PROP, 47%) and IL (VEH, 50%; PROP, 71%) that exhibited reductions in firing rate immediately after shock tended to maintain those decreases over the 1-hour recording session. These findings suggest beta-noradrenergic receptors are importantly involved in stress-induced modulation of neuronal activity in mPFC, and may contribute to cognitive deficits, including impairments in extinction learning.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01MH065961

Title: Renewal of extinguished fear induces Fos in ventral hippocampal neurons projecting to the medial prefrontal cortex

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Abstract: We have previously shown that anatomical disconnection of the ventral hippocampus (VH) and medial prefrontal cortex (mPFC) impairs the renewal of extinguished fear in rats (Orsini et al., 2011). Here we examined whether subpopulations of neurons in the ventral hippocampus (VH) that project to the mPFC, including the prelimbic cortex (PL) and infralimbic cortex (IL), are selectively or differentially engaged by the extinction and renewal or fear to an extinguished auditory conditioned stimulus (CS). Rats were ipsilaterally injected with two distinct fluorescent retrograde tracers into the IL and PL prior to behavioral testing. After recovery from surgery, the animals underwent standard fear conditioning and extinction procedures consisting of five tone (2kHz, 10sec, 80dB)-footshock (2s, 1mA) trials and 45 CS-alone trials in a novel context, respectively. Twenty-four hours later they rats received a retrieval test (5 CS-alone trials) in either the extinction context or a context different from that in which extinction occurred. All animals were sacrificed 90 min after the final test and freezing behavior served as the index of fear. Presentation of the CS outside of the extinction context induced fear renewal, relative to CS presentations inside the extinction context. Ventral hippocampal neurons were found to project to both IL and PL (PL projecting-neurons were more numerous) and a small number of neurons projected to both regions. Fos expression was similarly elevated in each subpopulation of mPFC-projecting neuron in animals tested outside the extinction context relative to those tested in the extinction context or home controls. Interestingly, this pattern of results is not consistent with circuit models appealing to different VH-mPFC circuits underlying the suppression and renewal of fear after extinction. Rather, these data suggest that projections from the VH to the mPFC have a unique role in fear renewal, and may not be involved in or

required for IL-mediated suppression of fear after extinction. Supported by a grant from the NIH (R01MH065961).

Disclosures: Q. Wang: None. **S. Maren:** None.

Poster

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Support: NIH Grant R01MH065961

Title: Does β-adrenergic blockade in the medial prefrontal cortex or basolateral amygdala rescue the immediate extinction deficit?

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Abstract: Post-traumatic stress disorder (PTSD) is often characterized by deficits in an affected person's ability to "extinguish" traumatic fear memories. Extinction involves learning that a fear-inducing cue or situation is safe after experiencing it repeatedly without harm. After fear conditioning in rats, presentation of an auditory conditioned stimulus (CS) many times in the absence of footshock (unconditioned stimulus, US) produces extinction of fear that is manifest as a reduction in freezing behavior. Interestingly, recently acquired fear is resistant to extinction--an immediate extinction deficit (IED) that has parallels with the extinction impairments in PTSD. Recent work in our laboratory has shown that systemic administration of propranolol (10mg/kg), a non-specific beta adrenoceptor antagonist, immediately before immediate extinction ameliorates the IED. This effect was not a retrograde disruption of fear memory consolidation, but rather an anterograde facilitation of fear extinction. In order to assess the specific neuroanatomical substrates mediating these effects, we infused propranolol (5ug) into the infralimbic cortex (IL) or basolateral amygdala (BLA) before immediate extinction training. Rats were implanted with unilateral (IL) or bilateral (BLA) cannulae and submitted to a standard fear conditioning procedure (5 CS-US trials). Fifteen minutes after conditioning, the rats were transported to a procedure room and infused with propranolol or vehicle. Immediately after the infusion, the rats were placed in a novel-conditioning chamber where they underwent an immediate extinction procedure (45 CS-alone trials); additional control groups were placed in the

chambers but did not undergo extinction training. Preliminary results suggest that infusions of propranolol into the mPFC do not affect immediate extinction, and the results of the BLA experiments are forthcoming. We expect that these experiments will ultimately localize the critical locus in the brain supporting the extinction-enhancing effect of systemic propranolol.

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Poster

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Title: Enhancement of traces of extinction memory to prevent fear return via up-regulation of infralimbic protein kinase M ζ

Authors: *Y. XUE[1], H.-B. HAN[2], Z.-Z. ZHU[2], J.-H. DENG[3], J.-L. YANG[4], L. LU[5];
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Abstract: Background: Extinction training has been suggested as a therapeutic strategy to inhibit excess fear. Extinction does not directly erase the existing memory, and the extinguished fear often remerge due to reinstatement, renewal, and spontaneous recovery. However, the cellular mechanism underlying the deficit in retention of extinction memory is unknown. Protein Kinase M zeta (PKMzeta) is a constitutively active atypical protein kinase C (PKC) isozyme. It has been showed to be required for maintaining long-term memories. Here, we explored that whether infralimbic PKMzeta influences the retention of extinction memory via modulating the strength of extinction memory trace. Methods: We trained rats to learn auditory fear conditioning, and microinjected LV-PKMzeta-GFP or LV-DNPKMzeta-GFP to genetic modulation the activity of PKMzeta. Results: Firstly, we found that PKMzeta levels was increased in the groups of fear conditioning and fear extinction in the BLA, whereas PKMzeta protein level was increased in the

IL in the fear extinction group. Then we found genetic inactivation of PKMzeta activity had no effect on the acquisition of extinction but blocked the retention of extinction memory. Furthermore, we found overexpression of PKMzeta in IL promoted the extinction training and prevented the reinstatement, renewal and spontaneous recovery of fear memory. Lastly, we found glutamine, dopamine, and norepinephrine modulated the extinction trace to erase fear memory via up-regulation of infralimbic PKMzeta levels. Conclusions Take together, our data provide evidence that the infralimbic PKMzeta controls the strength of extinction memory, and up-regulation infralimbic PKMzeta can erase fear memory via enhancement of extinction memory trace.

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Poster

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Title: The role of perineuronal nets and Nogo signaling in auditory cortex-mediated fear learning

Authors: ***S. B. BANERJEE**[1], V. A. GUTZEIT[2], R. C. LIU[1], K. J. RESSLER[2];
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Abstract: In recent years, cortical regions have been implicated in auditory fear learning and memory. Although it is clear that synaptic and neural changes occur in auditory cortex during fear learning, the molecular mechanisms that underlie these changes and allow learning associated plasticity to occur have not been elucidated. Here we hypothesized that neural signaling cascades, recruited during cortical developmental plasticity, play an important role in plasticity in the adult auditory cortex to mediate fear learning associated with auditory cues. We have begun to elucidate the role of the Nogo signaling pathway and its interaction with perineuronal nets (PNNs) in auditory fear learning within the auditory cortex. PNNs comprising of protein moieties and chondroitin sulfate proteoglycans (CSPGs) are components of the

extracellular matrix, are inhibitory for axonal sprouting and experience-dependent plasticity. The organization of CSPGs into PNNs coincides with the end of the visual cortex critical period and is delayed by dark rearing. Furthermore, the enzymatic degradation of CSPGs reactivates cortical plasticity in adulthood. Nogo receptor signaling plays a similar inhibitory role in visual cortex plasticity. In mice that have mutations in the Nogo-66 receptor, plasticity during the critical period continues abnormally into adulthood. Although little is known regarding the downstream signaling mechanisms of PNNs, a recent study has demonstrated that chondroitin sulfate moieties of PNNs can bind to the Nogo receptor, giving rise to the possibility that they interact and synergistically allow for inhibition of cortical plasticity. Here we tested our hypothesis that PNNs play a key role in auditory fear learning via Nogo signaling. We predicted that PNNs would be decreased in the auditory cortex after auditory fear conditioning along with Nogo signaling enabling the consolidation of auditory fear memories. We first observed that Nogo and Nogo receptor mRNA levels were decreased in the auditory cortex 2 hours after fear conditioning. Additionally, we found that mRNA levels of Brevican (a protein moiety of PNNs) were enhanced at this 2-hour time point. Furthermore, the disruption of PNNs prior to fear conditioning led to decreased fear expression 24 and 48 hours later. Together these data suggest that Nogo, NogoR, and PNNs are all dynamically regulated during the fear consolidation time window and they might be necessary for the normal consolidation of memories. We are in the process of testing the prediction that Nogo signaling is downstream of PNNs and that the interaction between moieties of the PNNs and Nogo receptor signaling is key for fear learning.

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Poster

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Title: Cortical perineuronal nets, parvalbumin neurons, and fear inhibition in adolescent rats

Authors: *K. D. BAKER, A. R. GRAY, R. RICHARDSON;
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Abstract: Perineuronal nets (PNNs) are extracellular matrix structures that preferentially surround mature GABAergic neurons which express the calcium-binding protein parvalbumin (PV). It has been suggested that aberrant PNN formation and PV neuronal maturation in humans may contribute to psychological disorders, many of which often emerge during childhood and adolescence. Experiment 1 investigated the normative developmental trajectory of PNN formation in the medial prefrontal cortex (mPFC) in juvenile (P24), adolescent (P35-36), and adult (~P70) rats. Dual-immunofluorescence staining was used to visualize the PNNs, using a fluorescent Wisteria floribunda agglutinin [WFA], and the expression of PV. There was a marked increase in the number of PNNs in both the prelimbic and infralimbic regions of the mPFC across the transition from the juvenile to adolescent period. The majority of PNNs in the mPFC surrounded PV neurons. Although there were no differences in the number of PV neurons across age groups, adolescent and adult rats had more PNNs surrounding PV neurons than juveniles. Experiment 2 examined the behavioral effect of impairing the maturation of prefrontal parvalbumin neurons through repeated exposure to an NMDA receptor antagonist in early adolescence. Specifically, we examined the effect of chronic MK801 on fear inhibition and PV neurons in the mPFC of adolescent rats. Adolescent rats received daily injections of MK801 (0.1 mg/kg), or saline, for 5 consecutive days from P27-P31. Rats were trained to fear a white-noise CS, given two days of extinction training, and tested for retention of extinction on consecutive days beginning at P34. Saline-treated rats showed good retention of extinction after two days of extinction training. In contrast, exposure to MK801 in early adolescence caused impaired fear inhibition despite extensive extinction training. That is, MK801-treated rats exhibited poorer extinction learning on day 2 which contributed to higher levels of fear at test. MK801 exposure reduced the number of PV neurons, but not PNNs, in the infralimbic cortex, a region implicated in fear inhibition. These findings suggest that the juvenile to adolescent developmental period is an important time for the formation of PNNs and the maturation of prefrontal PV inhibitory neurons. Further, they demonstrate that reduction of prefrontal PV inhibitory neurons is associated with markedly impaired fear inhibition in adolescent rats.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Sex differences in the effects of adolescent ethanol exposure on fear behavior in adulthood and corresponding structural changes in the medial prefrontal cortex

Authors: *T. GRUENE, C. REY, R. SHANSKY;
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Abstract: The adolescent brain is undergoing rapid developmental changes making it vulnerable to long-term effects of alcohol. The medial prefrontal cortex (mPFC) undergoes remodeling in adolescence and exposure to ethanol during this sensitive time in rats has been shown to impact mPFC dependent behaviors in adulthood. Specifically, repeated ethanol exposure in adolescent male rats has been shown to affect fear conditioning and extinction in adulthood. It is unclear, however, if ethanol exposure has similar effects in females. Fear conditioning and extinction paradigms are widely used to study the neurocircuitry underlying post-traumatic stress disorder (PTSD), which is twice as common in women. Thus, it is important to understand how factors contributing to vulnerability in the fear circuit affect females. To address this question the current study investigates sex differences in the effects of adolescent ethanol exposure on fear conditioning and extinction in adulthood. Adolescent female and male rats received i.p. injections of an ethanol solution starting at postnatal day 28 every three days for a total of five injections. As a control, a separate group of animals received saline injections following the same time course. After the last injection on postnatal day 40, animals were allowed to reach adulthood undisturbed until the first day of behavior testing in adulthood. At postnatal day 62 the animals were tested on a three day auditory fear conditioning and extinction paradigm. Fear was measured by freezing behavior and the results were analyzed for sex-specific effects of adolescent ethanol exposure. Additionally, we analyzed dendritic morphology in the mPFC to look for structural differences that map onto behavioral differences. Neurons in the mPFC were microinjected with Lucifer Yellow, allowing for visualization of dendritic morphology with confocal microscopy. Tracings of apical dendrites were analyzed for differences in arborization, and dendritic segments were analyzed for spine density and morphology. Studying sex differences in fear behavior and mPFC morphology following adolescent ethanol exposure has important implications for understanding how risk factors contribute to vulnerability in both males and females.

Disclosures: **T. Gruene:** None. **C. Rey:** None. **R. Shansky:** None.

Poster

746. Fear and Aversive Memories: Cortex

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DOD/CIMIT W81XWH-07-2-0011 to MJW

Title: Impaired fear expression but not extinction after controlled cortical impact in mice

Authors: *D. SIERRA-MERCADO[1,2], L. M. MCALLISTER[3], C. C. LEE[3], M. R. MILAD[3], E. N. ESKANDAR[2], M. J. WHALEN[3];
[1]Anat. & Neurobio., Univ. Puerto Rico Sch. of Med., San Juan, Puerto Rico; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Post-traumatic stress disorder (PTSD) is characterized in part by impaired extinction of conditioned fear. Traumatic brain injury (TBI) is thought to be a risk factor for development of PTSD. We examined the possible influence of TBI on conditioned fear and extinction in mice. We used a controlled cortical impact (CCI) model of cerebral contusion and a model of Pavlovian fear conditioning and extinction. To mimic the scenarios in which TBI occurs prior to or after exposure to an aversive event, unilateral (uCCI) or bilateral CCI (bCCI) was delivered to the parietal cortex at one of two time points: 1) prior to fear conditioning, or 2) after conditioning. Delay auditory conditioning was achieved by pairing a tone with a foot shock in "context A". Extinction training involved the presentation of tones in a different context (context B) in the absence of foot shock. Test for extinction memory was achieved by presentation of additional tones alone in context B the following day. In pre- or post-injury paradigms, uCCI did not influence fear learning and extinction. However, bCCI impaired conditioned fear, but not extinction. Impaired fear was not attributable to differences in locomotor activity or elevated plus maze testing. Our results demonstrate that bCCI, but not uCCI, impairs the acquisition and expression of conditioned fear. Together, the data suggest that severe contusion TBI does not contribute to PTSD by effects on extinction memory. Rather, our findings suggest that bilateral contusion may be maladaptive by impairing the ability to learn fear.

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Poster

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NHMRC grant APP1031688

Title: d-Cycloserine facilitates fear extinction in adolescent rats by increasing medial prefrontal cortex activation

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Abstract: Adolescents, both humans and rodents, exhibit a marked impairment in extinction of learned fear relative to both younger (e.g., juvenile) and older (e.g., adult) groups. This impaired fear inhibition could be caused by the medial prefrontal cortex (mPFC) not being recruited as efficiently during fear extinction in adolescents. For example, adolescent rats do not show the same extinction-induced increases in phosphorylated mitogen activated protein kinase (pMAPK)/extracellular signal regulated kinase (ERK) in the infralimbic cortex (IL) that are observed in juvenile and adult rats. The NMDA receptor partial agonist D-cycloserine (DCS), which enhances exposure therapy in humans with anxiety disorders, overcomes the extinction retention deficit in adolescent rats. The present experiments investigated the effects of DCS on the mPFC and amygdala by measuring pMAPK-immunoreactive (ir) neurons using immunohistochemistry. Adolescent rats were trained to fear a white-noise CS in one context followed by extinction in a second context or equivalent context exposure only (i.e., no extinction). DCS (15 mg/kg, s.c.), or saline, was administered systemically immediately after extinction training or no extinction. Separate groups of naive adolescent rats also received DCS or saline injections. Rats were tested for fear to the CS in the extinction context. Immunohistochemistry revealed that DCS combined with extinction training increased the number of pMAPK-ir neurons in the mPFC, with a similar effect being observed in the prelimbic (PL) and IL regions. This increased mPFC activity was not observed in naive animals given DCS. Furthermore, an opposite effect was observed in animals which received fear conditioning but no extinction, with DCS-treated animals showing reduced pMAPK expression in the PL and IL compared to those given saline. In contrast to the mPFC, no significant differences in levels of

pMAPK expression between any of the groups were detected in the amygdala, including the basolateral, central, and medial nuclei. In summary, the increased activation of MAPK signaling pathway in the mPFC by DCS suggests that it facilitates extinction retention in adolescent rats by enhancing activation of NMDA receptors in the mPFC. The present findings provide insight into the neural mechanisms underlying a pharmacological approach for improving fear inhibition in this vulnerable age group.

Disclosures: R. Richardson: None. **G.P. McNally:** None. **K.D. Baker:** None.

Poster

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Support: NIH Grant MH093412

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Brain and Behavior Research Foundation

Title: A+/B- fear discrimination and conditioned inhibition of freezing require NMDA receptor in posterior insular cortex

Authors: *A. R. FOILB[1], J. G. FLYER-ADAMS[2], S. F. MAIER[2], J. P. CHRISTIANSON[1,2]; [1]Psychology, Boston Col., Chestnut Hill, MA; [2]Univ. of Colorado, Boulder, CO

Abstract: Veridical detection of safe versus dangerous cues is critical to survival. We reported that the posterior insular cortex (IC) is critical for the stress-mitigating effect of safety signals on later anxiety behavior. Here, rats received "A+/B-" fear discrimination training in a standard fear-conditioning box over 5 days. 24 h after training rats were given a summation test in the training context in which freezing, an index of fear, was assessed during exposure to the context alone, A, A and B in compound (summation) and B alone. Importantly, rats rapidly learn to discriminate between A and B, yet B only becomes a conditioned inhibitor after several training sessions. We then show that intra-IC NMDA-receptor antagonist D-AP-5 (6μg/side) completely prevented both A+/B- discrimination and conditioned inhibition (AB) learning. These data suggest that IC is an important substrate of discriminatory fear learning.

Disclosures: **A.R. Foilb:** None. **J.P. Christianson:** None. **J.G. Flyer-Adams:** None. **S.F. Maier:** None.

Poster

746. Fear and Aversive Memories: Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-130393

Title: Regulation of discriminative fear conditioning by distinct prefrontal cortex subregions

Authors: *P. T. PIANTADOSI, S. B. FLORESCO;
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Abstract: Expressing appropriate discriminative fear responses towards aversive versus innocuous stimuli is an adaptive behavior. The neural substrates underlying fear learning have typically been studied using Pavlovian fear conditioning tasks, entailing exposure to a single conditioned stimulus (CS) that predicts an aversive stimulus. Dissociable roles for prefrontal cortex (PFC) subregions in the expression and extinction of conditioned fear have been reported. However, less is known about the circuitry underlying appropriate fear discrimination in the presence of an aversive conditioned stimulus (CS+) and one that is explicitly neutral (CS-). Here, we examined the contribution of two subregions of the rat medial PFC, the prelimbic (PL) and infralimbic (IL) cortex, to the acquisition and recall of discriminative fear conditioning. Male Long Evans rats were trained to lever press for sucrose reward on a VI-60 reinforcement schedule. They were then subjected to discriminative fear conditioning entailing eight, 30s presentations each of a CS+ (9kHz tone and flashing houselight + 0.5mA/0.5s footshock) and a CS- (1kHz tone and continuous cue lights, no shock). Two days later, rats underwent a test session entailing 4 presentations each of the CS- and CS+. Suppression of lever pressing during CSs served as the index of conditioned fear. Rats received inactivation (baclofen/muscimol; 75 ng each/0.3µl) or saline infusion prior to the conditioning or test phase of the task. Control rats displayed appropriate discrimination, suppressing lever pressing during the CS+ exclusively. Inactivation of PL during conditioning had no affect on the appropriate expression of discriminative fear. However, inactivation prior to retrieval completely eliminated suppression in response to the CS+, consistent with the integral role for PL in the expression of fear observed in single conditioned stimulus studies. In comparison, inactivation of IL prior to conditioning led to

reduced fear to the CS+ without altering CS- suppression during test. These results point to dissociable roles for PL and IL during the acquisition and recall of discriminative fear conditioning, suggesting that both regions support the appropriate allocation of emotional meaning to conditioned stimuli.

Disclosures: P.T. Piantadosi: None. **S.B. Floresco:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: ARC FT114666

Title: Pharmacogenetic excitation of medial prefrontal neurons mimics positive prediction error to enable fear learning

Authors: *J. YAU[1], G. MCNALLY[2];
[2]Psychology, [1]Univ. of New South Wales, Sydney, Australia

Abstract: Prediction error, or the discrepancy between actual and expected outcomes, is fundamental for Pavlovian conditioning. To determine the causal role of the dorsomedial prefrontal cortex (dmPFC) in fear prediction error, we used DREADDs to combine pharmacogenetic manipulation of dmPFC neurons with a behavioural associative blocking design. Rats received dmPFC application of either AAV5-SYN-hM3Dq-eYFP or AAV5-SYN-eYFP. 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. Immediately prior to Stage II, rats received i.p. injections of clozapine-N-oxide (3mg/kg; CNO) or vehicle. Blocking was shown by less fear to CSB at test relative to a control group that received Stage II but not Stage I training. AAV5-SYN-eYFP rats showed blocking of fear learning to CSB regardless of whether they were treated with CNO or vehicle prior to Stage II. However, AAV5-SYN-hM3Dq-eYFP rats showed attenuation of blocking at test when treated with CNO during Stage II, but not with vehicle. Thus, pharmacogenetic excitation of dmPFC neurons prevented blocking of fear learning. The prevention of blocking by pharmacogenetic manipulation of dmPFC neurons was not due to a change in processing the shock US, an increase in asymptotic levels of fear learning or augmentation of fear memory consolidation, because CNO did not affect the

acquisition of fear in simple CS - US pairings and did not affect the expression of fear in rats previously trained to fear a CS paired with either a low or higher intensity US. These results show that manipulations that increase activity of dmPFC neurons mimic positive prediction error to enable fear learning.

Disclosures: J. Yau: None. **G. McNally:** None.

Poster

746. Fear and Aversive Memories: Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: DOE to NSLS De AC02-98Ch10886

Title: Differences in distribution of zinc in frontal and hippocampal regions in B6 and 129 mice

Authors: *P. A. KAKALEC[1], C. GROEBER[2], R. TAPPERO[3], J. FLINN[2];
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Abstract: Proper distribution of zinc is critical to normal functioning of the brain. The 129S1/SvImJ (129S1) substrain of mice show deficits in the extinction of learned fears (Hefner et al., 2008; Camp et al., 2009) which can be rescued by a zinc deficient diet (Whittle et al., 2009). These data suggest the impairment could be due to an increased concentration of zinc. To understand better what structures are being affected by this increase in zinc content, and also to detect potential shifts in the distribution of zinc, we used microprobe synchrotron x-ray fluorescence microscopy (μSXRF) to analyze the levels of and distribution of zinc in the brain, examining the prefrontal and hippocampal regions. The data was collected on beamline X27A at Brookhaven National Laboratory. Data from the 129S1 mice were then compared to that from C57Bl/6N (B6) mice. Differences in distribution were seen in the infralimbic (IL) and prelimbic (PL) prefrontal regions. However, the greatest differences were seen in the hippocampus, which has a high level of zinc in the dentate gyrus, and is important in the formation of memories. Here the ratio of zinc in the dentate gyrus, compared to the CA1 region, of the 129 mice was ~ half of that for the B6s. Our data suggests that the 129S1 mouse, when compared to the B6, shows a redistribution of zinc within the Il , PL and the hippocampus, while its overall content remains constant between the two types of mouse.

Disclosures: **P.A. Kakalec:** None. **C. Groeber:** None. **R. Tappero:** None. **J. Flinn:** None.

Poster

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Support: CSUS SSIS/UEI Funding

Title: Perirhinal cortex involvement in delay fear conditioning to a discontinuous light stimulus

Authors: J. R. MOOTZ, C. A. CALUB, A. G. BECKNER, S. J. RUIZ, *S. C. FURTAK; Dept. of Psychology, California State Univ., Sacramento, CA

Abstract: Previous studies have shown that pretraining lesions to the perirhinal cortex (PER) impair delay fear conditioning to complex auditory cues, such as pre-recorded rat ultrasonic vocalizations (Lindquist, Jarrard, & Brown, 2004). In particular, the discontinuous nature of ultrasonic vocalizations was found to depend upon PER processing during delay fear conditioning to these stimuli (Kholodar-Smith, Allen, & Brown, 2008). The current experiment examined whether pretraining PER lesions induced similar impairments in delay fear conditioning to a discontinuous visual stimulus (conditioned stimulus; CS). In this study, Sprague-Dawley derived Albino male rats were broken into two groups: Lesion and Sham. The Lesion group underwent surgery and received intracranial injections of NMDA to produce bilateral excitotoxic lesions targeted at PER, while the Sham group underwent a similar surgical procedure with the exception that no injection was made. Following recovery, all subjects were trained on a three-day fear conditioning paradigm. Day 1, Fear Acquisition, consisted of 5 presentations of the CS (discontinuous light) paired with the unconditioned stimulus (US), a foot shock. The subsequent two days consisted of a Context Test and Light Test in counterbalanced order. In the Context Test the rat was placed back into the conditioning chamber for 10 mins with no CS or US presentations. In the Light Test the rat was placed into a new chamber (a context shift) and presented with the light CS with no US presentations for 6 mins. Freezing behavior (no movement except that necessary for breathing) was monitored and recorded throughout the experiment. Results show animals with PER lesions froze significantly less than Sham animals to the discontinuous light CS during the Light Test. Additional histological analysis will be necessary to eliminate the possibility that amygdala damage contributed to the observed deficit. These results further support PER in the processing of discontinuous stimuli

and extend this hypothesis to include modalities outside of audition, in particular stimuli within the visual modality.

Disclosures: **J.R. Mootz:** None. **C.A. Calub:** None. **A.G. Beckner:** None. **S.C. Furtak:** None. **S.J. Ruiz:** None.

Poster

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Support: CONACYT grant 128259

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Title: Changes in dendritic spine morphology and density in medial prefrontal cortex neurons associated to long term memory after different training intensity conditions

Authors: *M. E. TORRES GARCÍA[1], A. C. MEDINA[1], G. L. QUIRARTE[1], G. FLORES[2], R. A. PRADO-ALCALÁ[1];
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Abstract: Memory consolidation is the process by which newly learned information is stabilized into long-term memory. Particularly memory consolidation of inhibitory avoidance (IA) training requires the participation of medial prefrontal cortex (mPFC). The dendritic spines on pyramidal cells of mPFC represent the main postsynaptic elements of cortical excitatory synapses and they are fundamental structures in learning and memory. Thus, changes in these structures could reveal changes in the strength of these synapses and their connectivity with other structures. It has been shown that over-training protects against the amnesic effects of typical amnestic treatments. These findings support the suggestion that brain nuclei involved in memory processing are functionally connected in series during memory consolidation and that, after an enhanced learning experience, these structures become functionally connected in parallel. To determine if the intensity of training induces differential structural changes in layer 3 of mPFC, we performed a morphometric analysis in brain slices stained with the Golgi-Cox impregnation

method. Independent groups of male Wistar rats (250-350 g) were trained in IA using a low (1.0 mA) or a high foot-shock intensity (3.0mA) and were compared with a caged control group. Twenty-four h later, their retention latencies were measured, they were sacrificed immediately afterwards, and brain tissue was processed. Our preliminary results show an increase in the total dendritic spine density which depends on the intensity of training. There is also an increase of large mushrooms spines, which are known as 'memory spines'. Together, these data strengthen the link between cortical structural remodeling and memory consolidation of an enhanced learning experience.

Disclosures: M.E. Torres García: None. **A.C. Medina:** None. **G.L. Quirarte:** None. **R.A. Prado-Alcalá:** None. **G. Flores:** None.

Poster

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Title: Muscarinic acetylcholine receptor subtype-specific antagonism in retrosplenial cortex impairs contextual fear acquisition and retrieval

Authors: *K. LEADERBRAND[1], K. CORCORAN[2], J. WESS[3], S. TONEGAWA[4,5], J. RADULOVIC[2];
[2]Psychiatry and Behavioral Sci., [1]Northwestern Univ., Chicago, IL; [3]Lab. of Bioorganic Chem., Natl. Inst. of Diabetes and Digestive and Kidney Dis., Bethesda, MD; [4]Biol. and Brain and Cognitive Sci., MIT, Cambridge, MA; [5]RIKEN-MIT Ctr. for Neural Circuit Genet., Picower Inst. for Learning and Memory, Cambridge, MA

Abstract: Contextual fear conditioning is a model of anxiety disorders, such as post-traumatic stress disorder (PTSD), which are characterized by aberrant fear learning and memory retrieval. The retrosplenial cortex (RSC) is a brain region with reciprocal connections to known members of the fear circuit, such as hippocampus and prefrontal cortex, and is overactive in PTSD sufferers. Our lab has recently found that muscarinic acetylcholine receptors (mAChRs) in RSC

are critical for contextual fear acquisition, as well as retrieval. Here, we have utilized mAChR-subtype-specific antagonists and conditional knock-outs to demonstrate the specificity of these effects. Determining how mAChRs modulate memories of aversive events is critical for developing a complete model of and potential therapies for anxiety disorders.

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Poster

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Topic: F.02. Animal Cognition and Behavior

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Title: Pathway-specific corticoamygdala mediation of fear extinction

Authors: *O. BUKALO, C. R. PINARD, M. L. REGER, H. C. BERGSTROM, G. COLACICCO, E. F. BUSCH, A. HOLMES;
Lab. of Behavioral and Genomic Neurosci., NIH/NIAAA, Rockville, MD

Abstract: Delineating the functional contribution of corticoamygdala circuits to fear extinction in rodents has been the subject of intense study in recent years. Prior studies have shown that reciprocal connections between the infralimbic cortex (IL) and basolateral amygdala (BLA)/intercalated cell masses (ITC) are activated and exhibit plasticity during the formation and retrieval of fear extinction memories. However, while IL inputs to the amygdala are commonly theorized to mediate fear extinction, this remains to be definitively demonstrated, empirically. Therefore, we tested for alterations in fear extinction following pathway-specific manipulation of glutamatergic projections from the IL to the amygdala. To bidirectionally control this pathway in a temporally, spatially and cell-specific manner, adeno-associated virus containing either channelrhodopsin-2 or archaerhodopsin-3 was bilaterally infused into the IL (e.g., Cho et al. 2013; Senn et al 2014) and light bilaterally shone on IL-terminals in the amygdala, to stimulate or silence IL inputs. The pattern of innervation of virally-infected IL neurons in the BLA was examined by visualizing eYFP fluorescence and immuno-labeling ITC cells with FoxP2, and conducting *in vivo* electrophysiological recordings of neuronal activity. To test for the necessity of IL-amygdala inputs for fear extinction in C57BL/6J mice, IL-inputs were

silenced during each of 50 extinction training trials, and then testing for extinction expression the following day via a 5-trial retrieval session in which no light was shone. Next, to test whether recruitment of the IL-amygdala pathway was sufficient to facilitate or rescue extinction, IL-inputs were stimulated during extinction training trials in C57BL/6J and (extinction-impaired) 129S1 mice. Taken together, these findings could help advance our understanding of the neural circuits mediating fear extinction, with possible implications for elucidating the neuropathology of impaired extinction in anxiety disorders.

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Poster

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Human Frontiers Postdoctoral Fellowship (MAC)

Friedman Brain Institute and the Fishberg Department of Neuroscience (RLC)

Title: mPFC-BLA microcircuit involvement in fear conditioning

Authors: *M. ARRUDA-CARVALHO, R. L. CLEM;
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Abstract: Aversive memories are extraordinarily robust and long lasting, often leading to fear and anxiety disorders which prove resistant to treatment. Basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) exhibit abnormal activity as well as functional connectivity in fear-related disorders, and have long been established as critical regions for bidirectional fear regulation. In particular, fear expression and inhibition are associated with cue-related firing in the prelimbic (PL) and infralimbic (IL) subdivisions of mPFC, respectively. Although it is known that BLA and mPFC exhibit profound reciprocal connectivity, characterization of functional synaptic transmission in these pathways and its modulation by emotional learning is lacking. Here, we investigated synaptic properties of mPFC-BLA circuits through *ex vivo* optogenetics and whole cell patch clamp in mice. We found that fear conditioning elicited

pathway-specific synaptic strengthening in BLA principal neurons. Our findings confirm mPFC-BLA circuit involvement in the storage of fear memories and provide evidence that this is not a generalized function within mPFC. In order to further investigate this microcircuit and its impact on fear memory expression, *in vivo* optogenetic manipulation of mPFC neurons and their BLA axon terminals was also performed in conjunction with fear memory recall.

Disclosures: M. Arruda-Carvalho: None. **R.L. Clem:** None.

Poster

746. Fear and Aversive Memories: Cortex

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Topic: F.02. Animal Cognition and Behavior

Support: Research Growth Initiative from UW-Milwaukee

NIA

Title: Trace fear conditioning and extinction differentially modulate intrinsic excitability of mPFC neurons that project to the amygdala

Authors: *C. SONG[1], V. L. EHLERS[1], J. C. AIKTEN[1,2], T. BULA[1], J. R. MOYER, Jr.[1,2];
[1]Psychology, [2]Biol. Sci., Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Although neuronal activity in medial prefrontal cortex (mPFC) influences the expression of long-term conditioned fear memories, few studies have investigated mPFC contributions to the acquisition and extinction of trace fear memories (where CS and US are separated by a trace interval). Thus, the present study employed a combination of retrograde tracing and whole cell recordings from behaviorally characterized rats to evaluate neurophysiological changes in mPFC neurons that project to the amygdala. Rats received a unilateral infusion of a fluorescent retrograde tracer into BLA and were then randomly assigned to naïve, pseudoconditioned (PSEUDO), trace conditioned (TRACE), trace conditioned-retention (TRACE-RET), trace conditioned-extinction (EXT), or trace conditioned extinction-retention (EXT-RET) groups. Within 1-hr following testing in a novel chamber, brain slices were prepared and whole-cell recordings were made from fluorescently labeled layer 5 neurons in both infralimbic (IL) and prelimbic (PL) mPFC. Analysis of regular spiking neurons (121 IL, 87 PL) indicated that trace fear conditioning significantly enhanced intrinsic excitability of IL-BLA

projection neurons, via a reduction in spike threshold and activation of h-current. In contrast, intrinsic excitability was decreased in PL-BLA projection neurons through a decrease in input resistance. In both IL and PL, the conditioning-induced plasticity was significant only in COND rats, suggesting it was learning-specific. Furthermore, the learning-induced effect was transient, lasting up to 10 days after conditioning and was reversed by EXT or EXT-RET. In addition, the intrinsic excitability was positively correlated with behavioral performance in IL-BLA projection neurons following conditioning, but negatively in PL-BLA projection neurons after extinction, suggesting distinct roles of IL and PL neurons in trace fear memory expression. Trace fear conditioning significantly increased the input resistance of burst spiking neurons in PL, which enhanced their intrinsic excitability. This effect was reversed by EXT or EXT-RET. These data suggest that trace fear conditioning and extinction differentially modulate the intrinsic excitability of mPFC-BLA projection neurons in a subregion- and cell type-specific manner.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Research Growth Initiative from UW-Milwaukee

NIA

Title: Layer- and region-specific differences in the neurophysiological properties of medial prefrontal cortical neurons

Authors: *J. R. MOYER, JR.[1,2], V. L. EHLERS[1], C. SONG[1];
[1]Psychology, [2]Biol. Sci., Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Medial prefrontal cortex (mPFC) is critical for expression of long-term conditioned fear, and two subregions of the mPFC (prelimbic, PL and infralimbic, IL) play distinct roles during conditioned and extinguished fear memory retrieval. Our lab previously demonstrated that in adult rats, layer 2/3 (L2/3) neurons in IL are more excitable than PL neurons, and that this difference is diminished during aging, which may underlie our observed aging-related deficits in

extinction of a trace fear memory (Kaczorowski et al., 2012). We reason that such area-specific heterogeneity in intrinsic neuronal excitability may underlie the different roles of IL and PL during trace fear memory retrieval. Thus, the intrinsic properties of mPFC neurons in both L2/3 and L5 were studied from experimentally naïve adult rats. Analysis of the data from 108 neurons revealed that in both IL and PL, L2/3 neurons were more hyperpolarized than L5 neurons ($p < 0.01$ for both IL and PL neurons) and that the RMP was significantly correlated with somatic distance from the pial surface ($p < 0.01$ for neurons in both IL and PL). In addition, L5 neurons had significantly larger depolarizing sags than L2/3 neurons in response to hyperpolarization ($p < 0.01$ for both IL and PL neurons), suggesting more HCN channels are expressed in L5 neurons. This was confirmed by bath application of an HCN channel blocker - ZD7288, which significantly hyperpolarized and abolished the sag in L5 neurons ($p < 0.01$) but not L2/3 neurons. Furthermore, L2/3 neurons within PL were less excitable than L5 neurons, as they fired fewer action potentials in response to depolarizing current injections even when held at the same membrane potential (-67 mV; $p < 0.05$). This laminar difference was not observed in IL, where L2/3 neurons fired a comparable number of spikes as L5 neurons when they were held at -67 mV. Interestingly, however, these same L2/3 neurons fired less spikes when studied at rest (RMP = -75 mV; subset of 4 IL neurons, $p < 0.05$). Finally, although within L2/3, IL neurons were more excitable than PL neurons ($p < 0.01$), this dichotomy between IL and PL was not observed in L5 neurons. Further analyses indicated that the different intrinsic excitability between IL and PL in L2/3 is associated with a lower spike-threshold ($p < 0.05$) and higher input resistance ($p < 0.01$) in IL neurons. These data suggest that L5 neurons are more excitable than L2/3 neurons throughout the mPFC, and that IL neurons are more excitable than PL neurons in L2/3 but not in L5. Within mPFC, such layer- and subregion-specific differences in intrinsic excitability may underlie the distinct roles of IL and PL in the expression and suppression of a trace fear memory.

Disclosures: J.R. Moyer, Jr.: None. **V.L. Ehlers:** None. **C. Song:** None.

Poster

746. Fear and Aversive Memories: Cortex

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 746.26/TT1

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: VA grant 1I01BX001978-01

NIH grant R21MH083188

Title: A predictive model of PTSD: pre-existing functional differences in perirhinal and agranular insular cortex

Authors: *K. BUNTING[1,2], G. PEREZ[2], R. NALLOOR[2], A. VAZDARJANOVA[1,2];
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Abstract: Post-Traumatic Stress Disorder (PTSD), whether classified as an anxiety disorder, a stress disorder, or a trauma-associated disorder, has several hallmark components: intrusive memories, avoidance, hyperarousal, and altered mood and cognition. PTSD may best be described as a dysfunctional and unique interplay of memory and chronic physiologic stress. Only a portion of people who experience the same traumatic event develop PTSD; this is evidence of individual susceptibility. We have developed a unique behavioral model of PTSD in rats which allows us to classify animals as Susceptible or Resistant to developing PTSD-like behaviors prior to trauma. By testing anxiety and startle responses after a mild stressor, we identify individual animals who will show elevated startle response and delayed fear extinction after contextual fear conditioning. By predicting PTSD-like susceptibility before a traumatic event, we can examine pre-existing functional differences between Resistant and Susceptible rats, including differences in encoding of both traumatic and emotionally neutral events. Utilizing cellular compartment analysis of temporal activity using fluorescence *in situ* hybridization (catFISH) with the immediate-early genes Arc and Homer1a, we have previously reported impaired function in hippocampus and prefrontal cortex. Here we report that these alterations extend to other cortical areas, namely perirhinal and posterior agranular insular cortex. These findings suggest that pre-existing differences in susceptible individuals are more widespread than previously anticipated.

Disclosures: K. Bunting: None. **G. Perez:** None. **R. Nalloor:** None. **A. Vazdarjanova:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 747.01/TT2

Topic: F.02. Animal Cognition and Behavior

Support: Baylor University Research Committee and the Vice Provost for Research

Title: Prenatal stress increases fear conditioning and unconditioned anxiety in adult rats

Authors: *N. B. KEELE, M. A. MEYER, P. D. FOSS, M. L. MCREYNOLDS;
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Abstract: Mood and anxiety disorders are a major public health concern. Early life stress alters the developmental trajectory of limbic structures such as the amygdala, and contributes to increased vulnerability of developing these disorders. The purpose of these experiments is to investigate the effect of stress during prenatal development on amygdala-dependent and anxiety-like behavior in the adult offspring. Pregnant dams experienced a variety of stressors during the last week of gestation (including cold stress, restraint stress, and forced swim stress) three times each day at unpredictable times. Control dams were undisturbed during gestation, except for normal husbandry procedures. Stress dams did not differ from control dams in weight on G20, and there were no differences in litter size. Similarly, pups experiencing prenatal stress (PNS) were not different than unstressed controls (USC) in birth weight, length, nor anogenital distance. Beginning on postnatal day 30 (P30), one pup from each litter was chosen to determine the effect of prenatal stress on emotional and social behavior, and biochemical responses to acute stress. On P30, fear conditioning to a cue was increased in PNS offspring (33.4±12.5%, n=11) relative to the USC group (19.6±11.9%, n=11), but there was no difference in contextual conditioning condition (USC = -2±11%, n=11; PNS = 4±10%, n=11). On the elevated-plus maze, PNS rats made fewer open-arm entries (USC = 8±1; PNS = 5±1) and spend less time in the open arms (USC = 68±10s; PNS = 45±17s). No difference in anxiety-like behavior was observed in the open-field test. There were no differences between offspring of the two stress groups in either locomotor behavior, nor social behavior measured in the three-chamber apparatus. Serum corticosterone levels were measured by ELISA to determine the effects of prenatal stress on endocrine function in P30 offspring. Unexpectedly, baseline (unstressed) serum corticosterone concentration was lower in PNS rats (11 ± 10 pg/mL, n=3) that in USC rats (120 ± 61 pg/mL, n=4). However, PNS offspring showed a blunted, but prolonged corticosterone response to mild electric foot-shock stress (1mA, 1s). Together these data suggest that exposure to stress during the prenatal period changes behavioral measures of fear learning and anxiety in the adult offspring. Using this model, we may be able to determine biological effects of stress that increase vulnerability to anxiety disorders and perhaps understand better the biological basis of resilience to mental health disorders.

Disclosures: **N.B. Keele:** None. **M.A. Meyer:** None. **P.D. Foss:** None. **M.L. McReynolds:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 747.02/TT3

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH093950

Title: Fear conditioning circuitry in rats continues to emerge throughout periweaning period

Authors: A. D. DEAL[1], K. E. ERICKSON[1], *M. A. BURMAN[2];
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Abstract: Research over the past several decades has identified the third and fourth week of life as a time of critical development for the fear conditioning circuit in rats. Recent behavioral findings by our lab have suggested that both auditory and contextual fear continue to develop through PD 23. However, some previous research has indicated that fear expression to auditory and contextual cues differ in their emergence during development, concluding that fear expression to an auditory cue emerges between PD 15-17, while fear expression to a contextual cue does not emerge until PD 22-23. (i.e. Rudy 1992, 1993; Stanton 2000). In order to address these conflicting data, the present experiments further examine the emergence of fear conditioning during this developmental period using immediate early gene (IEG) expression to elucidate the functional emergence of the developing fear circuit neurobiology. In order to determine the structures involved with these behaviors, we used two different methods to look at IEG expression in four brain regions believed to have a role in auditory and contextual fear: the amygdala, perirhinal sulcus, hippocampus, and hypothalamus. The activity levels of these four regions during fear conditioning were quantified using qRT-PCR to measure the mRNA levels of IEGs FOS and EGR-1 and immunohistochemistry to determine the protein levels of FOS, quantified using unbiased stereology methods. Tissue was collected after fear conditioning on either PD 17 or PD 23/24 or after an auditory or contextual fear test on the following day. Our main conclusions are that both contextual and auditory fear conditioning continue to strengthen between PD 17 and PD 24, and that the pattern of immediate-early gene activation in limbic system structures during conditioning and retrieval of contextual memory changes during this developmental period. These results differ from the previous hypothesis that neural structures responsible for auditory conditioning, such as the amygdala, are largely mature by PD 17 and instead suggest that the interactions between the neural structures involved in the fear conditioning circuit are more complicated than we previously believed.

Disclosures: **A.D. Deal:** None. **M.A. Burman:** None. **K.E. Erickson:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

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Program#/Poster#: 747.03/TT4

Topic: F.02. Animal Cognition and Behavior

Support: T32MH096678-01

NARSAD Young Investigator Award

Title: Experience-dependent plasticity in parvalbumin interneurons disinhibits the amygdala fear circuit after emotional learning

Authors: *E. K. LUCAS, R. L. CLEM;
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Abstract: A delicate balance between excitation and inhibition must be maintained for proper circuit functioning. Within the prototypical fear circuit, discrete associative cues activate the lateral amygdala (LA), which then relays information to the central nucleus either directly or indirectly via the basal amygdala (BA). Previous work has demonstrated that auditory fear conditioning enhances excitatory transmission in LA pyramidal cells. Although local inhibitory interneurons in the LA and BA also receive direct synaptic input conveying associative cues, the contribution of these cells to fear memory encoding is not well understood. Using whole-cell patch recordings, we measured spontaneous and miniature inhibitory postsynaptic currents (IPSCs) in LA pyramidal cells and observed a significant reduction in IPSC frequency (but not amplitude or kinetics) after auditory fear conditioning. Given that the parvalbumin (PV) subclass of interneurons exerts the most potent inhibitory control over pyramidal cells, we targeted PV cells as a potential source of the fear-induced IPSC reduction. We next measured miniature excitatory and inhibitory postsynaptic currents in LA and BA PV cells and found that the ratio of excitation to inhibition in PV cells was decreased after fear conditioning in both nuclei. In LA, this shift was mediated by reduced mEPSC frequency; however, in BA, mIPSC frequency was increased. These results suggest that both pre- and postsynaptic plasticity contribute to attenuation of PV interneuron transmission after fear learning. We hypothesize that this experience-dependent disinhibition of the amydgala fear circuit serves to enhance encoding or consolidation of emotional memories. Experiments are currently underway to investigate the behavioral significance of PV cell activity through *in vivo* chemicogenetic manipulations. Our data demonstrate that one mechanism by which emotional memory tips the excitatory:inhibitory

balance in favor of excitation is through experience-dependent downregulation of PV cell transmission.

Disclosures: E.K. Lucas: None. **R.L. Clem:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant R01MH087755 to SN

Title: Development of neuronal single cell models for network simulations

Authors: *C. FRANKLIN, V. GUNTU, S. NAIR;
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Abstract: A challenge in computational neuroscience is to develop neuronal models with minimal complexity to enable the development of large networks of brain regions to understand the functions they might implement. Biologically realistic neuronal models aim to replicate the electrical functioning of the cell at the level of ionic channels, and sometimes even include complex dendritic trees and intracellular molecular cascades. Such models are computationally intensive and not suitable for implementation in networks. Reduced order models, such as the one proposed by Izhikevich (2007), aim to preserve the key neurocomputational properties and so form an attractive alternative for implementation in large network models. A systematic methodology is proposed to convert a biologically realistic neuronal model to an equivalent reduced order Izhikevich model given the key morphological features that impact network structure, while maintaining passive properties and fast and slow current dynamics. For instance, multiple compartments may be required, afferents may be distributed differently between basal and apical dendrites, channels may be distributed differently, afferent connectivity from short and long range sites may be different, and the synaptic plasticity mechanisms may vary between dendritic sites. At the same time, the implementation should ensure that the key neurocomputational properties of the unit are preserved. We used two network models with biologically realistic cells as benchmarks for comparisons of single cell model behaviors. For this, we embed the proposed single cells models into the network and compare performance with the original performance using the more detailed biologically realistic cells. The closer the

network performance is to the original case, the better the reduced order single cell model. One such benchmark network model is a 100-cell biologically realistic network model of lateral amygdala neurons that replicated the overall behavior of single units *in vivo* during a fear conditioning protocol (Li et al., 2009). Accordingly, we developed reduced order 3-compartment models of lateral amygdala neurons using the methodology proposed. Network performance in this case matched well with that of the original network. We will next compare performance against a 500-cell biologically realistic hippocampal network model. Quantification of comparison, including of the differences between the network model outputs and their causes, as well as of the differences in simulation times will be reported.

Disclosures: C. Franklin: None. **V. Guntu:** None. **S. Nair:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant R01-MH083710 to DP

NIMH grant R01-MH087755 to SN

Title: Central amygdala model integrates intra-amygdalar inputs during fear conditioning

Authors: *P. S. SAMARTH[1], D. PARE[2], S. NAIR[1];
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Abstract: Inputs about the conditioned (CS) and unconditioned (US) stimuli converge in the lateral amygdala (LA) during classical fear conditioning (FC). This leads to potentiation of CS inputs to LA neurons, and consequent increases in firing of central medial amygdala (CeM) cells. In turn, CeM cells drive conditioned fear responses via their projections to fear effector neurons. However, transmission of information from LA to CeM neurons is indirect, involving a multi-layered intra-amygdaloid network that includes basal amygdala (BA), the central lateral (CeL) nucleus, and intercalated cells (ITC), all of which project to CeM. Although consensus is emerging that CeM is the main fear output station of the amygdala for conditioned fear responses, details of connectivity within Ce are not well understood. LA axons contact CeL but

not CeM cells, and it is not clear how LA influences CeM through CeL. Also, CeL contains two types of CeM-projecting cells, with inhibitory (CeL-Off; PKCδ+) and excitatory (CeL-On; PKCδ-) responses to the CS. These two cell types are reciprocally connected by GABAergic synapses and it was hypothesized that the inhibition of CeL-Off cells by Cel-On cells leads to the disinhibition of CeM cells. However, it is not clear whether LA inputs connect differently to the two CeL cell subtypes and whether intrinsic and afferent synapses to Ce can undergo activity-dependent plasticity. For instance, it has been suggested that the connections between CeL sub-types could potentiate as a result of FC. We developed a 500-cell biophysical model of CeL and CeM to investigate how Ce might integrate inputs from LA, BA and ITC during FC. Inputs from LA, BA and ITC are modeled as spike trains using experimental tone response data at three specific time periods: habituation, after FC, and after extinction. We developed model Ce neurons that reproduced their experimentally observed physiological properties and spontaneous activity. In the network, 8% (19/240) of the model CeL cells showed CS-evoked increase and 11% (28/240) showed CS-evoked decrease in firing rates during habituation, matching experimental observations. The model performance during the fear protocol is being tuned using experimentally observed CeL and CeM tone responses. We are investigating whether endowing different types of intrinsic and extrinsic Ce synapses with the ability to undergo activity-dependent plasticity can reproduce these findings. We will also explore whether a winner-take-all competition underlies the formation of CeL-On and CeL-Off cells. In addition to providing insights into the role of Ce in regulating fear responses, such a model should also help generate testable predictions.

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Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant R01MH087755 to SN

Title: Comparison of single cell models for neuronal network simulations

Authors: ***A. ALTURKI**[1], A. NAIR[2], V. GUNTU[1], S. NAIR[1];
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Abstract: Biophysical conductance based models of neurons incorporate physiological information including morphology, current channels, and synapses. Such single cell models vary in complexity from those that have a single compartment to ones that have over 1000 compartments. Computational overheads necessitate the use of reduced order single cell neuronal models for large networks. However, in such cases, it is important that the reduced order models selected retain the key neurocomputational properties of biological cells. We compare single cell models with various compartments, 1, 2, 3, 5 and 69, for a rodent lateral amygdala principal cell by considering how well they match biological behaviors. Some of these include passive properties, current injection responses, subthreshold oscillation, synaptic responses, and the ability to provide balanced excitation and inhibition. We propose a methodology to develop a three-compartmental model that retains the important biological characteristics, and is suitable for incorporation into network models. We are presently investigating the performance of the single cell models by embedding them into an existing 100-cell network model of lateral amygdala neurons that reproduced experimentally observed emergence of two different tone responsive cell populations after a Pavlovian auditory fear conditioning paradigm. Preliminary results show that a single compartment model fails to reproduce passive and current injection properties simultaneously, whereas a 3-compartment model was successful. However, the 3-compartment model had limitations in integrating multiple synaptic inputs compared to the 69-compartment model. Although not much is known about the variation of current channel densities along the lengths of the dendrites, some biological reports are emerging in the literature that address this, and we will investigate this property also. The study should provide guidelines for the selection of appropriate single cell models that can provide a good compromise between biological realism and ease of computation when embedded in networks. Supported by NIMH grant R01MH087755 to SN.

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Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH grants R01MH083710 to DP

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Title: Competition among model lateral amygdala principal cells during Pavlovian fear conditioning

Authors: *F. FENG[1], S. PRANIT[1], D. PARE[2], S. NAIR[1];
[1]Electrical and Computer Engin., Univ. of Missouri-Columbia, Columbia, MO; [2]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ., Newark, NJ

Abstract: The CS responsiveness of principal neurons (PNs) in the dorsal subdivision of the rat lateral amygdala (LAd) is increased after auditory fear conditioning. In the most dorsal part of LAd, PNs display increases in CS responsiveness that last for only a few trials (transiently plastic or TP cells) whereas the more ventrally located PNs show a persistent increases in CS responses, even resisting extinction training (long-term plastic or LP cells; Repa et al.'01). A biophysical 1000-cell model of LAd (Kim et al., 2013a) successfully replicated the contrasting temporal patterns of increased tone responsiveness displayed by neurons in different parts of LAd. This model's features were constrained by prior experimental data, including neuromodulatory inputs, spatially heterogeneous intrinsic connections, activity-dependent synaptic plasticity and PNs with high to low spike frequency adaptation (Type A-C, respectively). The model was then used to investigate competitive mechanisms between PNs underlying the formation of the 'fear memory trace' in LAd (Han et al.'07,'09). These tests revealed that intrinsic excitability played an important role in determining whether PNs would be integrated in the fear memory trace: none of the fast adapting cells acquired increase tone responses to the CS as a result of conditioning. The model simulated the increase in intrinsic excitability resulting from CREB over-expression by converting a proportion of fast adapting PNs (type A cells) into slowly adapting ones (type C). Model runs of 25, 50, 75 and 100% CREB over-expression cases revealed that while LP cell numbers remained relatively constant across these cases, the numbers of TP cells varied widely. These results suggest that LP, and not TP cells, correspond to the fear memory trace cells reported by Han et al.'07. Also, two observations showed how plastic LP PNs band together to prevent plasticity in other PNs via disynaptic connections involving interneurons: after conditioning, reducing connection strengths between plastic LP PNs (new plastic cells) resulted in a 20% reduction in their CS responses and a 34% increase in the CS responses of non-plastic PNs. Also, disynaptic inhibition from plastic cells was lower for winners than losers for PNs. Last, the presence of tone, shock, and NM receptors were attributes that increased the chances of a PN becoming a winner in the competition.

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Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: EMBO ALTF 952-2011

NCCR Synapsy

Title: Central amygdala neurons gate expression of conditioned flight behavior

Authors: ***J. P. FADOK**, S. KRABBE, P. TOVOTE, P. BOTTA, C. XU, K. BYLUND, C. MUELLER, A. LUTHI;
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Abstract: Specific defensive fear behaviors evolved to avoid harm in the presence of fearful stimuli, such as predators. Although different fear responses are inborn, organisms must also use external cues to predict and respond to danger. Learning causes changes in neuronal circuits which allow stimuli to drive behavior that maximizes survival. Passive (freezing) and active (flight) responses are mediated via neural circuits in the amygdala (AMYG), ventromedial hypothalamus (VMH), the periaqueductal gray (PAG), and the nucleus basalis (NB). The intricate circuitry mediating freezing responses has been well established, however, the neural circuitry mediating conditioned flight responses is unknown. To explore this, we developed a novel behavioral paradigm in which mice alternate between freezing and flight responses based on the presentation of auditory cues within a serial compound stimulus. In this paradigm, mice maintain a strong conditioned freezing response to the pure tone component of the stimulus and a marked flight response only to the noise component. To explore whether single neuron activity in the AMYG correlates with cue-driven conditioned flight responses, we performed extracellular recordings in the central amygdala (CeA) of freely-moving mice during the conditioned flight paradigm. Remarkably, we observed neurons that develop responses to noise over the course of conditioning, and this noise-evoked increase in neuronal activity correlates with the active fear response. We performed Cre-dependent tract tracing studies and found that specific subtypes of neurons in the CeA project to the VMH, PAG, and NB, providing candidate populations for mediating conditioned flight. We targeted opsins to defined neuronal subpopulations with Cre-dependent vectors in order to manipulate active responses. Furthermore, we used optogenetics to identify specific neuronal subtypes during extracellular recordings in the conditioned flight paradigm. Finally, rabies-based retrograde tracing revealed that the CeA flight network receives selective input from the ventral hippocampus, thalamus, and hypothalamus. These findings point to a previously unexplored function for the CeA as an important regulator of active fear behavior.

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Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant R37MH57502

California National Primate Research Center RR00069

Title: Large-scale plasticity following neonatal amygdala lesions in the rhesus macaque

Authors: ***D. GRAYSON**[1], M. BUONOCORE[1], J. BENNETT[2], D. AMARAL[1];
[1]Psychiatry, [2]UC Davis MIND Inst., Sacramento, CA

Abstract: Introduction: Non-human primate models allow the investigation of crucial developmental proceses such as plasticity. Prior work has shown that rhesus monkeys demonstrate a critical period for behavioral recovery following damage to medial temporal lobe structures, although how the brain reorganizes itself remains unclear. In order to uncover these mechanisms, we have performed bilateral amygdala lesions in neonatal rhesus monkeys, followed by structural MRI (sMRI) and diffusion-weighted imaging (DWI) in these animals during adulthood. We hypothesized that, relative to control animals, these animals would exhibit increased volume of grey matter structures distant from the amygdala, as well as higher fractional anisotropy (FA) in pathways connecting these regions. **Methods**: Rhesus macaques underwent surgery at two weeks of age to receive bilateral amygdalectomies via neurotoxic injection (n=8) or sham treatment (n=8). T1-weighted sMRI were acquired at ages 4 and 12, and high resolution DWI at age 12. Study-specific average T1w and FA templates were constructed using ANTS. Voxelwise between-group comparisons were conducted for morphometric analysis using deformation-based morphometry (DBM). Voxelwise FA and RD were also statistically compared and overlaid with tracts defined via streamline tractography. **Results**: As expected, lesioned animals displayed contraction of temporal lobe gray matter surrounding the amygdala and in the anterior commissure ($p<.001$). On the other hand, we observed volume expansion covering the full extent of the cingulate cortex (CC, $p<.03$), which was stable across timepoints. The CC was also thicker and more variably folded in the lesioned animals. Analysis of DTI

images demonstrated increased FA in white matter lateral to the CC, and increased FA in tracts emanating from structures proximal to the amygdala, particularly the putamen. These data provide strong evidence for plasticity related to increased volume and white matter connectivity in multiple cortical and subcortical structures. **Discussion**: This work has demonstrated large-scale structural correlates of plasticity that are surprisingly widespread. It is known that macaque monkeys with neonatal amygdala lesions recover critical cognitive, social, and behavioral capacities, such as the fear-potentiated startle, whereas macaques that undergo amygdalectomy in adulthood do not. Our imaging findings may relate directly to these recovered behaviors, and reveal key features of the brain's ability to adapt to early injury or insult. Furthermore, this work underscores the utility of high-resolution DBM and DWI in identifying neural correlates of plasticity.

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Poster

747. Fear and Aversive Memories: Amygdala

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Neuroscience Training Program, UW-Madison

Title: Immunohistochemical characterization of somatostatin expressing neurons in the primate amygdala

Authors: *R. KOVNER[1], P. H. ROSEBOOM[1], D. A. FRENCH[1], J. A. OLER[1], A. S. FOX[1], J. L. FUDGE[2], N. H. KALIN[1];
[1]Psychiatry, Univ. of Wisconsin, Madison, WI; [2]Psychiatry, Univ. of Rochester Med. Sch., Rochester, NY

Abstract: The amygdala is a heterogeneous group of nuclei implicated in various emotion-related functions. The central nucleus of the amygdala (Ce) is an integral part of the neural

circuit mediating fear and anxiety and its altered function has been implicated in stress-related psychopathology. Recent studies indicate that the lateral division of the central nucleus (CeL) functions to gate fear expression through tonic inhibition of the medial division of the central nucleus (CeM). Our goal is to understand the complex interplay among neurotransmitter systems in the primate CeL. Studies in rodents demonstrate that the CeL is primarily composed of GABAergic neurons that can be divided into subtypes including somatostatin (SOM) expressing neurons and protein kinase C δ (PKCδ) expressing neurons. In rodents, some PKCδ neurons co-express corticotropin-releasing hormone (CRH), a neuropeptide linked to stress and anxiety. A population of neurons in the amygdala also expresses dopamine-and cyclic AMP-regulated phosphoprotein (DARPP-32), a marker of cells receiving dopaminergic input. Except for PKCδ, previous studies have characterized the distribution patterns of these markers in the monkey amygdala; however, there have been no reports of their co-localization. Describing the co-localization of these markers in primate CeL is critical to understanding the molecular interactions of these neurotransmitter systems in relation to understanding their putative role in mediating stress-related psychopathology. The present study, in macaques, sought to characterize neurons identified using NeuN, a neuron-specific marker, which co-express SOM with DARPP-32, CRH or PKCδ using triple-labeling immunofluorescence. Consistent with previous reports, we observed SOM expression in fibers and cell bodies in the CeL, where the fiber expression was particularly dense. However, we found that SOM expressing neurons comprise less than 5% of the total number of neurons in the primate CeL. Interestingly, approximately 30% of neurons expressing SOM co-express PKCδ, a finding inconsistent with rodent data, suggesting a species-specific difference. Approximately 50% of the SOM expressing neurons also expressed CRH but co-expression of SOM with DARPP-32 was not observed. These results suggest that SOM expressing neurons may also release CRH, but surprisingly do not appear to be directly modulated by dopaminergic input. This work provides a translational link between microcircuit data in rodents and human neuroimaging studies. Future experiments aim to identify cell populations in Ce involved in primate anxiety using RNA sequencing in conjunction with behavioral phenotyping.

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Poster

747. Fear and Aversive Memories: Amygdala

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Program#/Poster#: 747.11/TT12

Topic: F.02. Animal Cognition and Behavior

Support: Chinese government's China Scholarship Council

SNSF grant FN 31003A-138526

Title: Dynamics of the recruitment of Lateral Amygdala neurons following successive fear learning to different conditional stimuli

Authors: *R. NIU, M. ABATIS, R. STOOP;
Dept. of Psychiatry, CHUV, Prilly, Switzerland

Abstract: Recent work has shown that in the lateral amygdala (LA) only 25% of neurons are activated during fear learning, while their suppression erases the new fear memory. Further results suggest that pCREB increases the probability of recruitment in a competitive manner by increasing basic neuronal excitability (Han et al., Science 2007; 2009, Zhou et al., Nat Neurosci 2009). However, the mechanism behind this selection process and how these neurons are subsequently organized into a fear memory trace remains unknown. Assuming the formation of consecutive fear memories each recruits 25% of LA neurons, the question also arises whether there is an overlap in the specific recruitment of neurons, and whether this facilitates recruitment of subsequent series? Furthermore, what type of pre-training excitability predisposes neuronal recruitment, is it e.g. intrinsic excitability, sensitivity to the conditioned stimulus (CS) or sensitivity to connections from neighboring neurons? In this study we used a protocol of consecutive fear learning to two different CS stimuli to gain insight into how these different types of excitabilities (and changes therein induced by fear learning) affect neuronal recruitment into a fear memory trace. To monitor neuronal activity during fear learning, seven rats were implanted with 32 electrodes reaching into the LA. We recorded from 233 putative pyramidal neurons among a total of 256 neurons. In particular, individual pyramidal neurons could modulate their firing rate in response to the conditioned tone (conditioned neurons) or non-conditioned tones (generalizing neurons). Out of those 233 neurons, 86 (37%) were conditioned to one or both CSs. More precisely, we found 35 neurons conditioned to CS1 only, 25 conditioned to CS2 only and 26 conditioned to both CSs. Notably, in spite of a successful behavioral extinction, the firing rate of those conditioned neurons in response to the CS remained unchanged throughout memory testing. Fear conditioning also affected local functional connectivity, which increased from a basal 0.9% to 1.4% and 3.6% after the first and second learning, respectively. Furthermore, the pre-conditioning characteristics of the conditioned neurons could predict neuronal recruitment based on three factors: 1) initial sensitivity to auditory inputs, with tone-sensitive neurons being more easily recruited than tone-insensitive neurons; 2) baseline excitability levels, with more highly excitable neurons being more likely to become conditioned; and 3) the number of afferent connections received from local neurons, with neurons destined to become conditioned receiving more connections than non-conditioned neurons.

Disclosures: **R. Niu:** None. **M. Abatis:** None. **R. Stoop:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Program#/Poster#: 747.12/TT13

Topic: F.02. Animal Cognition and Behavior

Support: SNSF Grant FN 31003A-138526

Title: Characterizing connectivity and signal propagation in the lateral amygdala through local networks

Authors: ***M. ABATIS**[1], R. PERIN[2], H. MARKRAM[2], R. STOOP[1];
[1]Dept. of Psychiatry, CHUV, Prilly, Switzerland; [2]Lab. of Neural Microcircuitry, Brain Mind Inst., EPFL, Lausanne, Switzerland

Abstract: Fear conditioning combines an unconditioned stimulus with a conditioned stimulus (CS) so that the CS can subsequently elicit fear-related responses by itself. While plasticity of converging signals onto a single lateral amygdala (LA) neuron has been extensively studied to underlie this process, little is known about the role of LA sub-networks in fear-memory encoding. We hypothesize that fear signals are re-integrated in the LA through local neuronal assemblies. To study these, we used whole-cell patch-clamp recordings to simultaneously access up to 12 neurons in 74 acute, horizontal, brain slices of P15 rats (n = 30), with the aim of mapping network topology and enhancing our understanding of transmission and plasticity of LA-to-LA synapses. We recorded from 571 neurons, including 17 interneurons. Biocytin labeling revealed that neurons were not topographically clustered based on their electrophysiological phenotypes. Network connectivity was assessed by delivering, successively in each neuron, a train of 8 pulses at 20 Hz and monitoring for induced post synaptic potentials. We observed an overall connectivity of 2%, with 63 pyramidal-to-pyramidal neuron connections, 12 interneuron-to-pyramidal neuron connections and 6 pyramidal neuron-to-interneuron connections. The chance to observe a connection decreased with inter-somatic distance. This suggests that LA neurons are organized into a small-world rather than a scale-free network (Feldt et al., TINS, 2011). Plasticity was assessed in 13 connected pyramidal neurons by pairing 15 pre- and post- synaptic trains at 30 Hz, with a 10ms delay between pre- and post- synaptic stimulation. This led to a redistribution of the amplitude of EPSPs with a high-amplitude first response for 4 connections, which was transient unless 1 mM Glutamine, a Glutamate precursor,

was co-incubated. Since directions of LA-LA connections seemed to be random, we monitored the pathway of a signal propagating in the LA. To this end, we generated epileptiform bursts by incubating an isolated LA in 20 μM bicuculline and 5 mM K^+. The onset of spontaneous bursts was tracked in 3 clusters of 4 electrodes positioned at the caudal, medial and rostral edges of the LA. The burst spread from caudal to medial to rostral LA with a delay of 68±31ms between caudal and rostral ends. To summarize, we found that LA neurons are organized into a small-world network with 2% connectivity and while the directions of LA connections do not yield apparent patterns, a burst of epileptiform activity propagates from caudal to rostral direction, coincident with a caudal termination of external capsule fibers in the LA and rostral projections from LA to basolateral amygdala.

Disclosures: **M. Abatis:** None. **R. Perin:** None. **R. Stoop:** None. **H. Markram:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

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Program#/Poster#: 747.13/TT14

Topic: E.05. Stress and the Brain

Support: NWO-NIHC, project no. 056-23-011

Title: Altered functional architecture of amygdala-centered networks in adolescent posttraumatic stress disorder

Authors: ***M. AGHAJANI**[1], I. M. VEER[3], M. A. W. RINNE-ALBERS[1], M.-J. VAN HOOF[1], N. J. VAN DER WEE[2], R. R. J. M. VERMEIREN[1];
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Abstract: Objective: Posttraumatic stress disorder (PTSD) is a debilitating, prevalent, and difficult to treat psychiatric disorder characterized by a constellation of symptoms related to the experience of a traumatic event. Whereas multiple lines of research implicate amygdala-centered network dysfunction in the pathophysiology of adult PTSD, no study has yet examined the functional architecture of amygdala-centered networks in adolescent PTSD. Using intrinsic functional connectivity (IFC) analysis, we investigated functional connectivity of the basolateral (BLA) and centromedial (CMA) amygdala in sexually abused adolescents with chronic PTSD relative to matched controls. Methods: We employed seed-based correlation analysis to examine

the IFC of the BLA and CMA in 19 adolescents with chronic PTSD (age 16.2 ± 1.8) relative to 23 age, sex and IQ matched controls (age 15.5 ± 1.78). First, time courses from our regions of interest, the left and right BLA and CMA, were extracted. Then, the temporal correlation between these extracted signals and the signal from all other brain voxels was calculated in a general linear model for each hemisphere, with white matter, cerebrospinal fluid, and six motion parameters included as confound regressors. Volume censoring (aka scrubbing) was additionally applied to remove the effects of micro movements on our data. The resulting individual IFC maps of the bilateral BLA and CMA were then fed into a higher-level mixed effects analysis. Age, sex, and IQ were included in the analysis as covariates to correct for their possible confounding effects. All results were corrected for multiple comparisons using cluster-based correction ($p<0.05$, initial cluster forming threshold of $Z>2.3$). Results: Whole brain analysis revealed diminished right BLA connectivity with the ventromedial prefrontal cortex (vmPFC) and rostral anterior cingulate cortex (rACC) in PTSD patients. In contrast, our analysis revealed increased left CMA connectivity with the orbitofrontal cortex in PTSD patients. Noteworthy, in PTSD patients, diminished right BLA connectivity with the vmPFC and rACC predicted more anxiety and dissociation symptoms, measured at 3 and 6 months after scanning (all $p<0.05$). Conclusions: In line with neurocircuitry models of PTSD in adults, these findings suggest that amygdala-centered networks subserving cognitive and emotional functions are disorganized in adolescent PTSD. These results represent an important step towards characterizing the neurocircuitry of adolescent PTSD, thereby informing the development of predictive biomarkers and effective treatment strategies.

Disclosures: M. Aghajani: None. **I.M. Veer:** None. **M.A.W. Rinne-Albers:** None. **M. van Hoof:** None. **N.J. van der Wee:** None. **R.R.J.M. Vermeiren:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 747.14/TT15

Topic: F.02. Animal Cognition and Behavior

Title: ErbB4 modulates fear expression and anxiety through the central amygdala

Authors: *S. AHRENS, H. LI, M. PENZO, B. LI;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Anxiety disorders represent the most common class of psychiatric diseases with high lifetime prevalence. The amygdala is an important brain structure in the modulation of emotional responses, and dysfunctions in amygdala circuitries can lead to altered fear responses and changes in anxiety. Within the amygdala, the central lateral nucleus (CeL) has been shown to be involved in the learning and expression of conditioned fear as well as in anxiety-related behaviors. Consisting of exclusively GABAergic neurons, the CeL provides important regulative function over the central medial nucleus (CeM), the main output station of the amygdala, and also directly targets downstream effctors of fear responses in the brainstem. One major population of CeL neurons expresses somatostatin (SOM) and those neurons have been implicated in the storage of aversive memories. The schizophrenia-risk gene ErbB4 is expressed in GABAergic neurons within the amygdala. ErbB4 has been shown to control development and function of glutamatergig synapses in cortex and hippocampus, but its role in SOM+ neurons within the amygdala circuit is unknown. Here we show that conditional ErbB4-/- (KO) mice in which the ErbB4 gene is ablated in SOM+ neurons, display an intensified fear expression, increased generalization and impaired extinction of fear responses compared to their wildtype littermates in a classical auditory cued fear-conditioning paradigm. In addition, general anxiety, measured by the elevated plus maze task, was increased in these mice. Electrophysiological recording in brain slices revealed an enhanced glutamatergic synaptic transmission onto SOM+ neurons in the CeL of KO mice. Together, our results demonstrate that changes in the level of synaptic transmission onto SOM+ class of GABAergic neurons in the CeL can lead to changes in fear expression and anxiety and point to an important role of these neurons in the pathophysiology of anxiety disorders. In addition, our study uncovers a novel modulatory synaptic function of ErbB4 in a CeL circuit and sheds light on a mechanistic link between ErbB4 genetic defects, CeL circuit dysfunction, and abnormalities in emotional behavior.

Disclosures: **S. Ahrens:** None. **H. Li:** None. **M. Penzo:** None. **B. Li:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Program#/Poster#: 747.15/TT16

Topic: F.02. Animal Cognition and Behavior

Support: Conacyt 130524

PAPIIT-UNAM IN214111

PAPIIT-UNAM IN202414

Title: Glucocorticoid receptor activation in the amygdala and corticosterone release are related to stress levels during contextual fear conditioning

Authors: R. PONCE-LINA[1], N. SERAFIN[1], M. CARRANZA[2], J. N. PARGA-MARTÍNEZ[1], R. A. PRADO-ALCALÁ[1], M. LUNA[2], *G. L. QUIRARTE[1];
[1]Neurobiologia Conductual y Cognitiva, [2]Dept. de Neurobiología Celular y Mol., Inst. de Neurobiologia, UNAM, Queretaro, QRO, Mexico

Abstract: Glucocorticoids release during the learning process facilitates memory consolidation, especially of highly emotional or stressful experiences. This effect depends on several factors; among them are the glucocorticoid receptor (GR) activation in the basolateral amygdala (BLA) and the level of arousal level produced by the experience. The present work aimed to investigate the correlation between the stressful stimulus level applied during contextual fear conditioning (CC) training, the corticosterone (CORT) concentration in serum, and the percentage of active GR in the BLA. Male Wistar rats (250-350 g) were trained in CC in a single session (11 min) under different shock intensities (0.0, 1.0, and 3.0 mA). There were two groups for each intensity, in the first one the subjects were tested for retention at 48 h and then on two extinction sessions 24 h apart. The other group was sacrificed 15 min after training by decapitation, blood was collected and the amygdala extracted. CORT levels in serum were quantified by the ELISA method, and with amygdala samples the active GR (serine 232 phosphorylated) levels were measured by SDS-PAGE/WB. Rats trained with 1.0 and 3.0 mA intensities learned the task equally well, since the average freezing time increased in the post-shock session and were statistically different compared to the group trained with 0.0 mA. CORT levels were higher in the 3.0 mA group, while levels of GR in the amygdala of all groups have the same total GR density, but the active GR percentage increases relative to the control group. The above results indicate that in the CC test, the higher CORT levels are released during an aversive training, and there is the possibility that memory consolidation of CC depends on the number of activated GR in the amygdala. We acknowledge the technical assistance of Angel Mendez, Cristina Medina, Eneida M. Pérez, Martín García, Leonor Casanova and Lourdes Lara. This work was funded by CONACYT (Grant 130524, Scholarship RPL 342154) and PAPIIT-UNAM IN214111 and IN202414.

Disclosures: **R. Ponce-Lina:** None. **N. Serafin:** None. **M. Carranza:** None. **J.N. Parga-Martínez:** None. **R.A. Prado-Alcalá:** None. **M. Luna:** None. **G.L. Quirarte:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: NSF GRFP Grant DGE-0718124

Title: CRF produced in the central nucleus of the amygdala facilitates cue-dependent fear learning

Authors: *C. A. AKERS[1], J. SCHULKIN[2], M. S. CLARK[3], L. S. ZWEIFEL[4];
[1]Dept. of Pharmacol., [2]Dept. of Obstetrics & Gynecology, [3]Dept. of Psychiatry & Behavioral Sci., [4]Departments of Pharmacol. and Psychiatry & Behavioral Sci., Univ. of Washington, Seattle, WA

Abstract: Corticotropin-releasing factor (CRF) is a stress-associated peptide that is a key component in hypothalamic-pituitary-adrenal axis activation, but is also produced outside of the hypothalamus where it acts throughout the central nervous system. One of the major sources of extrahypothalamic CRF is the central nucleus of the amygdala (CeA). Direct manipulation of CRF expression in this region has been shown to modulate anxiety; however, the role of CRF and more broadly the role of CRF producing neurons in the regulation of fear processing remains poorly understood. To determine the role of CRF producing neurons in anxiety and cued fear, we selectively silenced these cells through conditional viral-mediated expression of tetanus toxin light chain (AAV1-DIO-TeTx$_L$GFP). Mice expressing Cre-recombinase under the endogenous promoter of the *Crh* gene were injected with AAV1-DIO-TeTx$_L$GFP into the CeA and subsequently tested on elevated plus maze and open field assays before and after cue-dependent fear conditioning. We find that CRF producing neurons are essential for cued fear learning but do not influence anxiety. To address the function of CRF specifically in the CeA, we genetically inactivated CRF in mice containing conditional *Crh* alleles ($Crh^{lox/lox}$) through viral-mediated delivery of Cre recombinase (AAV1-CreGFP). Similar to silencing CRF producing neurons, mice lacking the ability to synthesize CRF in the CeA also showed impaired cue-dependent fear learning but normal anxiety responses. These findings demonstrate that CRF synthesis and release is critical for the function of these neurons in cued fear learning.

Disclosures: C.A. Akers: None. **J. Schulkin:** None. **M.S. Clark:** None. **L.S. Zweifel:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: Wayne State Dept. Psychiatry and Behavioral Neurosciences

Wayne State MD/PhD Program

Title: Amygdala CRF and glutamatergic systems after fear conditioning and extinction: relationship to the magnitude of the unconditioned stimulus

Authors: *M. J. LISIESKI[1], H. S. YONO[2], A. DUDA[2], M. L. TOMASZYCKI[3], S. A. PERRINE[1];
[1]Psychiatry and Behavioral Neurosci., Wayne State Univ. Sch. of Med., Detroit, MI;
[3]Psychology, [2]Wayne State Univ., Detroit, MI

Abstract: It is well known that the amygdala is involved in the acquisition and expression and extinction of conditioned fear, and that corticotropin releasing factor (CRF) and glutamate signaling play essential roles in these processes. Less is known about how the magnitude of conditioned fear may be encoded in the amygdala. Thus, we used a cued fear conditioning and extinction paradigm with 3 different unconditioned stimulus (shock) intensities (0.2, 0.4, or 0.6 mA) to investigate the neurobiological correlates of magnitude of conditioned fear in the amygdala in adult male Sprague-Dawley rats. We found that fear behavior (freezing) increased as shock intensity increased, and that this relationship between freezing behavior and shock intensity persisted through extinction and extinction recall sessions. Following behavioral testing, brains were collected, sectioned, and analyzed for the number of cells immunopositive for CRF1 and glutamate NMDA receptors, as well as the co-localization of these proteins, in multiple regions of the amygdala. These measures allow us to characterize whether changes in glutamate and CRF signaling and their interactions within neurons are related to the magnitude of conditioned fear. These studies will provide a broader understanding of the role of the amygdala CRF and glutamatergic systems in encoding the magnitude of conditioned fear, a mechanism germane to understanding aspects of normal and pathological fear learning.

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Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant 1R15MH095032 - 01

Title: CRF receptor localization in the basolateral nucleus of the amygdala

Authors: K. CALAKOS, *E. P. BAUER;
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Abstract: The neuropeptide corticotropin-releasing factor (CRF) is released during periods of anxiety and can modulate learning and memory consolidation. Dysregulation of the secretion of CRF as well as chronic hyperactivation of the extrahypothalamic CRF system are implicated in anxiety disorders. The basolateral nucleus of the amygdala (BLA) is a critical structure for Pavlovian fear conditioning and extinction and contains dense concentrations of CRF receptors. A previous study showed that increasing levels of CRF in the BLA significantly impairs fear extinction. Here we investigated which neurons in the BLA express CRF receptors. Fluorescent immunocytochemistry was performed to determine if co-localization occurs between CRF receptors and target neurons of interest including excitatory and inhibitory neurons, as well as inhibitory subsets such as parvalbumin, and cholecystokinin-expressing interneurons. Our results show that there are dense concentrations of CRF receptors in the ventral portions of the BLA, with sparse staining in more dorsal areas. CRF receptors co-localize with alpha-CaMKII, a marker of the principal excitatory neurons of the BLA as well as with GAD67, an enzyme found in GABAergic inhibitory interneurons. Furthermore, these receptors are expressed on both parvalbumin- and cholecystokinin-expressing inhibitory neurons. How activation of these receptors affects neuronal activity is crucial to understanding the differential effects of CRF on fear conditioning and extinction.

Disclosures: K. Calakos: None. **E.P. Bauer:** None.

Poster

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Support: BMBF CSCC

BMBF Bernstein Fokus 01GQ0923

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DFG HHDP FOR 1738 GZ WI830/10-1

Title: Amygdala shrinkage and enduring cognitive impairment following systemic inflammatory disorder

Authors: M. BOEHME[1], S. SCHMIDT[1], R. CLAUS[2], J. REICHENBACH[3], C. GASER[4], *O. W. WITTE[1];
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Abstract: *Question:* The mechanisms causing prolonged cognitive impairments after severe systemic infections (e.g. sepsis) are not well understood. The pathophysiology is highly complex and widely affects almost all levels of brain processing. To identify vulnerable structures responsible for the long-term outcome, we screened the brain for temporal changes of brain morphology over time. *Methods:* Repetitive T2-weighted brain magnetic resonance images were acquired at baseline as well as at 1, 2, 4, 8 and 12 weeks following peritoneal lipopolysaccharide application (LPS, *E. coli* O127:B8) in male RccHan:WIST rats (3 months old) and processed by deformation-based morphometry (DBM). Sickness was scored and long-term behavioural abnormalities were investigated using the open field and the accelerated RotaRod test. Subsequently, changes in neuronal activity and microglia activation were examined by immunohistochemistry (Arc, Iba1) in cross-sectional experiments. *Results:* LPS induced severe sickness for about 4 days. Following recovery from this acute illness, rats showed an anxious/depressive phenotype and reduced endurance to sustain motor performance for at least 4 weeks. DBM revealed an acute whole brain volume loss of about -1 % and area-restricted gray matter shrinkage that evolved bilaterally in the basolateral amygdala (-11%) and the auditory cortex (-5%), persisted for up to 2 weeks and reached baseline levels after 4 weeks. This temporal shrinkage was associated with focally reduced neuronal activity and global activation of microglia. *Conclusions:* We emphasize the high potential of repeated neuroimaging as a research tool to assess fundamental structural predictors of impaired cognition. The trajectory of behavioural abnormalities was most significantly reflected by shrinkage and inactivation of the amygdala - the main gatekeeper of sensory information and key regulator of intrinsic vigilance. Their inhibition disrupts attentional networks and conscious perception of the environment and is therefore predestined to explain cognitive impairments following severe infectious diseases.

Disclosures: **M. Boehme:** None. **O.W. Witte:** None. **S. Schmidt:** None. **R. Claus:** None. **J. Reichenbach:** None. **C. Gaser:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: ISF

GIF

Title: EphrinA4 mimetic peptide targeted to EphA binding site impairs the formation of long-term fear memory in lateral amygdala

Authors: ***M. DINES**, R. LAMPRECHT;
Univ. of Haifa, Haifa, Israel

Abstract: Fear conditioning leads to long-term fear memory formation and is a model for studying fear-related psychopathologies conditions such as phobias and post-traumatic stress disorder (PTSD). Long-term fear memory formation is believed to involve alterations of synaptic efficacy mediated by changes in synaptic transmission and morphology in lateral amygdala (LA). EphrinA4 and its cognate Eph receptors are intimately involved in regulating neuronal morphogenesis, synaptic transmission and plasticity. To assess possible roles of ephrinA4 in fear memory formation we designed and used a specific inhibitory ephrinA4 mimetic peptide (pep-ephrinA4) targeted to EphA binding site. We show that this peptide, composed of the ephrinA4 binding domain, interacts with EphA4 and inhibits ephrinA4-induced phosphorylation of EphA4. Microinjection of the pep-ephrinA4 into rat LA 30 minutes before training impaired long- but not short-term fear conditioning memory. Microinjection of a control peptide derived from a non-binding E helix site of ephrinA4, that does not interact with EphA, had no effect on fear memory formation. Microinjection of pep-ephrinA4 into areas adjacent to the amygdala had no effect on fear memory. Acute systemic administration of pep-ephrinA4 1 hour after training also impaired long-term fear conditioning memory formation. These results demonstrate that ephrinA4 binding sites in LA are essential for long-term fear memory formation. Moreover, our research shows that ephrinA4 binding sites may serve as a target for pharmacological treatment of fear and anxiety disorders.

Disclosures: **M. Dines:** None. **R. Lamprecht:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Support: NIMH Grant R01 MH084966

U.S. Army Research Office and the Defense Advanced Research Projects Agency grant W911NF-10-1-0059

Title: Identification of neuronal networks encoding aversive and appetitive learning in the basolateral amygdala

Authors: ***S. CORREIA**[1], K. A. GOOSENS[2];
[1]McGovern Inst., MIT McGovern Inst., CAMBRIDGE, MA; [2]Dept. of Brain and Cognitive Sci., MIT-McGovern Inst. for Brain Res., Cambridge, MA

Abstract: Understanding the brain circuits that acquire motivated behaviors contributes to our basic understanding of how we learn, and is essential for understanding disorders associated with abnormal perception of reward and punishment, such as depression and post-traumatic stress disorder (PTSD). The basolateral amygdala (BLA) is involved in processing of both aversive and appetitive behaviors. Anatomically, this structure consists primarily of excitatory neurons that project to many different brain areas. These projection neurons are under strong inhibitory control from a small population of local GABAergic interneurons. Several studies suggest that fear conditioning decreases GABAergic drive within the BLA, and that fear extinction training remodels perisomatic inhibitory synapses located directly on BLA neurons activated by fear. We sought to determine whether the decrease in GABAergic drive following fear conditioning is a unique feature of aversive conditioning or if it is also present following appetitive conditioning. For this purpose, we trained rats on either a Pavlovian aversive or appetitive task, in which an auditory cue predicted either footshock (aversive) or sucrose (appetitive) delivery. After behavioral training we collected and processed the brain tissue for immunolabelling of the immediate early gene cFos and markers of inhibitory neurons in the BLA. Fluorescence confocal imaging was used to quantify the activation of inhibitory interneurons in the BLA. Our data shows decreased activation of parvalbumin positive interneurons in the BLA of animals that

underwent aversive conditioning but not animals that were trained on an appetitive conditioning task. These data suggests that valence determines the specific amygdala circuit engaged by learning.

Disclosures: S. Correia: None. **K.A. Goosens:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Topic: F.02. Animal Cognition and Behavior

Support: USC School of Medicine Research Development Fund

NIH R01-DA027305

Title: Generation of synchronized inhibition in BLa pyramidal cells by activation of mAChRs

Authors: *L. LIU, A. J. MCDONALD, D. D. MOTT;
Dept. of Pharmacology, Physiol. and Neurosci., Univ. South Carolina Sch. of Med., Columbia, SC

Abstract: Neurons in the basolateral amygdala (BLa) oscillate rhythmically during emotional processing. These oscillations are thought to be important to integrate sensory inputs, allow binding of information from different brain areas and facilitate synaptic plasticity in target downstream structures. The mechanism through which BLa neurons synchronize their activities is poorly understood. In hippocampus and cortex neuronal oscillations can be induced by acetylcholine acting on muscarinic receptors (mAChRs). The BLa receives dense cholinergic innervation from basal forebrain, providing a basis for mAChR regulation of oscillatory behavior in this region. The aim of this study is to define the role of mAChRs in generating synchronized firing of BLa pyramidal cells. Using whole cell recording in brain slices from 15-30 day old male Sprague-Dawley rats, we found that puff application of muscarine (2 s, 50 μM) induced theta frequency rhythmic IPSPs in BLa pyramidal cells. These IPSPs synchronized pyramidal neuron (PN) firing at theta frequencies. Rhythmic IPSCs were blocked by bicuculline or TTX, but not glutamate receptor antagonists (CNQX and D-APV), suggesting that they were produced by direct muscarinic depolarization of interneurons. Recordings from neurochemically-identified interneurons revealed that muscarine selectively depolarized parvalbumin (PV)-containing, fast

firing, but not PV, regular firing or somatostatin (SOM)-containing interneurons. This depolarization was mediated by M3 mAChRs. Dual cell recordings from connected interneuron-PN pairs indicated that action potentials in fast firing, but not regular firing interneurons were strongly correlated with large IPSCs in BLa PNs. Furthermore, selective blockade of M3, but not M1 mAChRs suppressed the rhythmic IPSCs in BLa PNs. These findings suggest that muscarine induces rhythmic IPSCs in PNs by selectively depolarizing PV, fast firing interneurons through M3 mAChRs. Furthermore, we found that rhythmic IPSCs were highly synchronized between PNs throughout the BLa. This synchronization required gap junctional coupling between interneurons when the two PNs were far apart. However, adjacent PNs could be synchronized by a single PV interneuron. Collectively, these studies provided a mechanism for cholinergic induction of theta oscillations in BLa.

Disclosures: L. Liu: None. **A.J. McDonald:** None. **D.D. Mott:** None.

Poster

747. Fear and Aversive Memories: Amygdala

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Program#/Poster#: 747.23/TT24

Topic: F.02. Animal Cognition and Behavior

Support: NIDA Grant SCIDA034995

Title: The role of the amygdala in timing and sensory-specific learning following reward devaluation in the peak procedure

Authors: *K. S. ELAYOUBY[1], H. M. NASSER[2], A. R. DELAMATER[2];
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Abstract: When learning to anticipate biologically important environmental events, such as the delivery of food reward, an organism comes to learn about and, presumably, encode both its specific sensory features, i.e., "what" it is, as well as its time of occurrence, i.e., "when" it occurs. Characterizing the nature of the reward representations and how we learn about them is important to properly investigate the neural mechanisms underlying learning. We used a peak timing procedure in order to dissociate sensory-specific, and temporal features by exploring reward devaluation effects, in both Pavlovian and instrumental paradigms. The data suggests that timing and sensory-specific learning processes can both be revealed with a US devaluation treatment in a peak timing task. The effects appear additive in the Pavlovian experiments but

interactive in the instrumental task. Using excitotoxic lesions we investigated the role of the amygdala in encoding sensory-specific features during a Pavlovian peak timing procedure. Preliminary results suggest that amygdala lesions, prior to training, attenuated devaluation effects with no influences on peak timing. This suggests that the amygdala is necessary for encoding sensory-specific, but not temporal features of reward.

Disclosures: K.S. Elayouby: None. **H.M. Nasser:** None. **A.R. Delamater:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 747.24/TT25

Topic: F.02. Animal Cognition and Behavior

Title: Comparison of inputs to active neurons in basolateral amygdala following different behavioral treatments

Authors: *L. DEBLANDER[1], H. FONTAINE[1], A. WEIBLE[1], H. WU[1], I. WICKERSHAM[2], C. KENTROS[1,3];
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Abstract: There are two general strategies for encoding information in the brain. In the labeled-line strategy, one neuron responds to only one stimulus and information about that stimulus is conveyed when the neuron begins firing. In the across-fiber pattern strategy, an entire neural population responds to a wide range of stimuli and information about a particular stimulus is conveyed in the patterns of neural activity across the population. We chose to investigate these two strategies using a connectomics approach, reasoning that, if a labeled-line strategy were employed, neurons activated by different behavioral treatments would have different retrograde inputs. Conversely, if an across-fiber pattern strategy were employed, neurons activated by different behavioral treatments would have the same retrograde connections. These neural coding strategies are most commonly mentioned in the field of sensory processing where control of stimulus parameters can help in distinguishing them. We chose to focus on the basolateral amygdala complex because it is a step above sensory processing in that it can create and maintain associations between neutral and aversive stimuli (LeDoux, 1995). Specifically, we asked if learning a simple association changes the inputs to and active neurons in basolateral amygdala when compared to neurons active during exploration of a familiar environment. To

address the question, we used a transgenic cross in combination with recombinant rabies to trace the monosynaptic retrograde connections of neurons in basolateral amygdala recently activated by tone delay fear conditioning or exploration of the same environment without delivery of the tone or shock. Ongoing experiments are aimed at identifying the molecular signatures of the primarily infected neurons and locating and quantifying retrograde infection.

Disclosures: L. Deblander: None. **H. Fontaine:** None. **A. Weible:** None. **H. Wu:** None. **I. Wickersham:** None. **C. Kentros:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 747.25/TT26

Topic: F.02. Animal Cognition and Behavior

Support: MH038774

Title: Facilitation and suppression of instrumental responding require connections between the lateral and central amygdala

Authors: *V. CAMPESE[1], R. GONZAGA[1], J. E. LEDOUX[1,2];
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Abstract: The encoding of Pavlovian associations between conditioned stimuli (e.g., tone: CS) and aversive unconditioned stimuli (e.g., shock: US) occur in the lateral amygdala (LA). Once acquired, the CS elicits conditioned responding in the form of defensive behaviors (e.g., freezing), which have been shown to depend upon the central amygdala (CeA). The trained CS is also capable of modulating learned instrumental behaviors. Whether the influence an aversive CS exerts on behavior is facilitative or suppressive depends on the motivational nature of the reinforcer associated with the instrumental response. For example, when an aversive CS is presented while the subject is responding for food, the CS suppresses instrumental behavior (i.e., conditioned suppression or cSup). Alternatively, after subjects have been trained to shuttle to avoid shock, an increase in shuttling rate is observed when the same aversive CS is presented (i.e., conditioned facilitation or cFac; Campese et al., 2013). Despite the opposing behavioral influences the CS exerts on responding, both the cSup and cFac effects depend on CeA (Killcross et al, 1997; Campese et al, 2014). In the current project, we show that serial LA-CeA

connections are required for cSup of licking for sucrose. LA and CeA lesions as well as disconnections of these structures eliminated cSup in these studies. We also present data from a task that measures both cSup and cFac within the same subjects using an identical response for both tasks (i.e., two-way shuttling). Preliminary findings show that CeA lesions eliminate both cSup and cFac. Future studies will determine how microcircuits in the CeA are responsible for the opposite behavioral effects produced by the aversive CS.

Disclosures: V. Campese: None. **R. Gonzaga:** None. **J.E. LeDoux:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 747.26/TT27

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Japanese Ministry of Education, Culture, Sports, Science and Technology grant (No.06770740)

Title: The role of amygdalar serotonergic neural system in fear memory

Authors: *T. IZUMI, Y. OHMURA, T. YOSHIDA, M. YOSHIOKA;
Neuropharm., Hokkaido University, Col. of Med., Sapporo, Japan

Abstract: The amygdala is a crucial brain structure for anxiety, and it is speculated that the serotonergic neural system in the amygdala has an important role in regulating fear memory. In our previous study, we indicated that systemic administration of selective serotonin reuptake inhibitor (SSRI) and local injection of SSRI into the basolateral nucleus of amygdala (BLA) attenuated freezing behaviors in conditioned fear in rats. In the present study, we investigated the effects of serotonergic terminal lesions in the amygdala by local injection of 5,7-dihydroxytryptamine (5,7-DHT), on memory-dependent and independent fear in rats. A 5,7-DHT lesion in the amygdala attenuated memory-dependent fear (freezing) assessed by the conditioned fear, but enhanced memory-independent fear (aversion to open space) assessed by the elevated plus-maze test. These results suggested that the role of the amygdalar serotonergic system in fear was different between memory-dependent and independent fear and, in particular, it is paradoxical that an amygdalar serotonergic lesion exerted a similar effect on memory- dependent fear to SSRI. On the other hand, attenuating effect of local injection of SSRI, citalopram, into BLA (3 μg/side) on freezing was dose-dependently blocked by local co-administration of 5-HT_{1A}

antagonist, WAY100635 (0.005-0.5 μg/side). From the above results, it is speculated that the amygdalar serotonergic system has a dual role, namely, one enhances and another attenuates memory-dependent fear, and that SSRI exerts anxiolytic effect on memory-dependent fear via 5-HT_{1A} receptors in BLA.

Disclosures: **T. Izumi:** None. **Y. Ohmura:** None. **T. Yoshida:** None. **M. Yoshioka:** None.

Poster

747. Fear and Aversive Memories: Amygdala

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 747.27/TT28

Topic: F.01. Human Cognition and Behavior

Support: Stress and Motivated Behavior Institute

Title: Amygdala lesions impair lever press avoidance acquisition of inbred Wistar-Kyoto rats, but not outbred Sprague Dawley rats

Authors: ***K. M. MOENCH**[1], D. P. MILLER[1,2], M. T. ALLEN[2,3], K. C. H. PANG[2,4], R. J. SERVATIUS[2,4,5];
[1]Psychology, Neurosci., Carthage Col., Kenosha, WI; [2]Stress and Motivated Behavior Inst., New Jersey Med. School, Rutgers, The State Univ. of New Jersey, Newark, NJ; [3]Sch. of Psychological Sciences, Col. of Educ. and Behavioral Sci., Univ. of Northern Colorado, Greeley, CO; [4]DVA, NJHCS, East Orange, NJ; [5]Joint Biomed. Engin. Program, New Jersey Inst. of Technol. and Grad. Sch. of Biomed. Sciences, Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Wistar-Kyoto (WKY) rats have been proposed as a model for anxiety vulnerability due to their observed behavioral inhibition coupled with their enhanced acquisition of signaled avoidance and resistance to extinction. The amygdala is critical for Pavlovian fear and threat, and instrumental conditioning with aversive outcomes. In our initial efforts to understand the role of the amygdala in signaled lever press avoidance, we demonstrated that lesions of the amygdala disrupted, but did not prevent, the acquisition of avoidance behavior in both WKY and SD rats. In that work, a lever press during the warning signal (WS) was reinforcement by an immediate cessation of the warning signal (WS), prevention of shock and initiation of the safety signal. These multiple reinforcement contingencies did not allow for an unambiguous interpretation of the role of the amygdala in learning. In this study we removed the contingency between the lever

press and WS termination. Avoidance prevented foot shock delivery, but only after the completion of the 60 sec tone signal regardless of when the avoidance occurred. Similar to previous reports, WKY sham rats showed the fastest acquisition and highest rate of avoidance whereas the SD sham rats displayed a balance between avoidance and efficient escape responses. In the absence of a response contingency in WS termination, amygdala lesions in SD rats did not appear to alter avoidance learning at all. In contrast, amygdala lesions impaired, but did not prevent avoidance acquisition of WKY rats. Anxiety disorders are best understood as a diathesis of inherent vulnerabilities and risk. The role of the amygdala in anxiety can be differentiated by examining avoidance acquisition between the strains.

Disclosures: K.M. Moench: None. **D.P. Miller:** None. **M.T. Allen:** None. **K.C.H. Pang:** None. **R.J. Servatius:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.01/TT29

Topic: F.02. Animal Cognition and Behavior

Title: The role of the basal forebrain cholinergic neurons in cued extinction memory

Authors: *W. B. SCHREIBER, S. KELLER, D. KNOX;
Univ. of Delaware, Newark, DE

Abstract: Fear extinction learning and memory requires inhibition of neural activity in amygdala (AMY) nuclei driven by neural substrates in the ventromedial prefrontal cortex (vmPFC), resulting in the behavioral phenotype of a decreased fear response (e.g. low levels of conditioned freezing). Fear extinction memory retrieval is sensitive to contextual feature manipulations, rendering extinction memory retrieval sensitive to hippocampal (HIPP) input to the AMY. Function of the vmPFC and HIPP requires cholinergic innervation from basal forebrain cholinergic neurons (BFCNs), including neurons in the nucleus basalis (NB), horizontal diagonal band of Broca (hDBB), vertical diagonal band of Broca (vDBB), and medial septum (MS). Given the importance of the vmPFC and HIPP for extinction memory, we hypothesized that intact BFCNs would be critical for extinction memory. We found that complete BFCN lesions using 192 IgG-saporin disrupted acquisition of cued fear extinction memory (Experiment 1). Follow-up studies examining more restrictive cholinergic lesions of the MS/vDBB (Experiment 2) or the NB/hDBB (Experiment 3) suggest these two clusters of BFCNs may differentially

modulate acquisition and retention of cued extinction memory. The overall results of this study suggest that BFCNs are a component of the fear extinction circuit and a potential target for the pharmacological treatment of psychological disorders thought to stem from extinction memory deficits (e.g. PTSD).

Disclosures: W.B. Schreiber: None. **S. Keller:** None. **D. Knox:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.02/TT30

Topic: F.02. Animal Cognition and Behavior

Support: ARC FT114666

Title: The effects of pharmacogenetic excitation or silencing of vlPAG on Pavlovian fear conditioning

Authors: *C. ARICO[1], G. P. MCNALLY[2];
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Abstract: The ventrolateral periaqueductal gray (vlPAG) has been implicated in predictive fear learning, specifically in contributing to encoding of fear prediction errors. However, it is unknown whether this role depends on excitation or inhibition of vlPAG neurons or whether specific subpopulations of neurons in vlPAG are important for this role. The present experiments investigated the role of the vlPAG in fear learning using excitatory and inhibitory DREADDs with a conditioned suppression measure of fear. Three experiments will be reported. In each experiment, rats were microinjected with AAV5-SYN-hM3Dq-eYFP or AAV5-SYN-hM4Di-eYFP, or AAV5-SYN-eYFP prior to the commencement of a fear conditioning. In Experiment 1, the effects of pharmacogenetic or pharmacogenetic inhibition of vlPAG on the acquisition of fear learning to a single visual CS paired with an aversive US were examined. Rats received injections of Clozapine-N-oxide (CNO) 30 min prior to conditioning. In Experiment 2, the effects of pharmacogenetic inhibition or excitation of vlPAG on learning in a blocking design were examined. Rats received Stage I fear conditioning of CSA whereas control animals received no training. In Stage II, all rats were presented with a compound of CSA + CSB paired with an aversive US. Rats received injections of Clozapine-N-oxide (CNO) 30 min prior to Stage II. Rats were tested the next day for fear responses to CSB. Finally, in Experiment 3, the effects

of pharmacogenetic inhibition or excitation of vlPAG on learning in an unblocking design were examined. Rats received Stage I fear conditioning of CSA-0.6mA US or CSA-0.9mA US. In Stage II, all rats were presented with a compound of CSA + CSB paired with a 0.9mA US. Rats received injections of Clozapine-N-oxide (CNO) 30 min prior to Stage II.

Disclosures: C. Arico: None. **G.P. McNally:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.03/TT31

Topic: F.02. Animal Cognition and Behavior

Support: ARC FT114666

Title: The roles of the lateral habenula and basolateral amygdala in punishment

Authors: *P. JEAN-RICHARD DIT BRESSEL[1], G. P. MCNALLY[2];
[1]UNSW, Kensington, Australia; [2]UNSW, Sydney, Australia

Abstract: Volitional behaviour that causes an aversive outcome will be suppressed, i.e. punished. The neural bases of punishment are poorly understood. Two regions implicated in punishment are the lateral habenula (LHb), which inhibits midbrain dopamine neurons in response to aversive stimuli, and the basolateral amygdala (BLA). The current experiments studied the roles of the LHb and BLA in punishment. During Stage I, rats were trained to respond on two individually-presented levers that caused the delivery of food pellets. During Stage II, one of these levers caused delivery of a footshock as well as pellets (punished lever) while the other lever continued to only deliver pellets (unpunished lever). Rats rapidly reduced responding on the punished lever over the course of Stage II. Infusions of the AMPA antagonist NQBX or baclofen/muscimol (BM) into the LHb had no effect on the acquisition or expression of this punishment, regardless of whether the levers were presented individually or together in a discrete choice test. However, NBQX into the LHb increased locomotion. Infusions of BM into the caudal BLA, but not rostral BLA, attenuated the acquisition and expression of punishment when the levers were presented individually but not during an unpunished choice test. These results indicate that the LHb is not necessary for, and that there is a rostral-caudal gradient in BLA contributions to, punishment.

Disclosures: **P. Jean-Richard Dit Bressel:** None. **G.P. McNally:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.04/TT32

Topic: E.05. Stress and the Brain

Title: Impairment of the basal ganglia dopamine system predicts an altered brain ageing in prenatally stressed rats

Authors: ***J. MARROCCO**[1], J. MAIRESSE[2,3], E. GATTA[2,3], H. BOUWALERH[2,3], M. CANNELLA[4], M. MOTOLESE[4], G. BATTAGLIA[4,3], A. PITTALUGA[5], P. CALABRESI[6], F. NICOLETTI[7,4,3], S. MACCARI[2,3];
[1]IRCCS Ctr. Neurolesi bonino Pulejo, Messina, Italy; [2]UMR 8576 Dept. of Glycobiology, Univ. of Lille 1/CNRS, Villeneuve d'Ascq, France; [3]LIA (International Associated Laboratory: Univ. of Lille1/CNRS, France; IRCCS Neuromed and Sapienza Univ. of Rome, Italy), France, Italy; [4]IRCCS Neuromed, Pozzilli, Italy; [5]Dept. of Pharm., Univ. of Genoa, Genoa, Italy; [6]IRCCS Fondazione Santa Lucia, Rome, Italy; [7]Dept of Physiol. and Pharmacol., Sapienza Univ. of Rome, Rome, Italy

Abstract: Stress, with the ensuing activation of the hypothalamic-pituitary-adrenal axis, is a major risk factor for neuropsychiatric disorders, such as depression, schizophrenia, and anticipated brain ageing. Stress-related disorders share common neurochemical and behavioral substrata that may crucially involve the dopaminergic system. This is sustained by findings showing that oscillation of dopamine (DA) concentration could be associated to non-motor disturbances, including cognition and depression. The animal model of prenatal restraint stress (PRS) in rats recapitulates the hallmark of anxious/depressive disorders. Indeed, PRS phenotype mainly reflects changes in glutamate and DA systems in hippocampus and basal ganglia motor circuit, respectively. To assess whether PRS have endurance effects on the programming of brain ageing, we characterized the striatal DA system and associated behavioral parameters of adult male PRS rats (3-4 month-olds) and aged PRS rats (20 month-olds), i.e. the adult/old offspring of dams exposed to repeated episodes of stress during pregnancy. We found that adult and aged PRS rats showed impairment in behavioral tasks indicative of striatal function, such as active avoidance, grip strength, pasta matrix, and ladder rung walking test. Interestingly, these tests have convincing predictive validity in animal models of Parkinson’s disease. Also, both adult

and old PRS rats displayed a marked reduction in depolarization-evoked 3H-DA release in superfused striatal synaptosomes. Microdialyses of striatal DA also revealed that adult PRS rats showed a reduction in DA release after veratridine-induced depolarization. An increased cAMP response to A2A adenosine receptor activation was found in striatal slices prepared from adult PRS rats; this was combined with an increase in the transcript encoding for A2A receptor in the striatum. Curiously, aged PRS rats showed a slight decrease in the striatal transcript of A2A receptor. Adult, but not aged, PRS rats also showed increases in the transcript encoding for pro-dynorphin, the precursor of dynorphins that restrain the activity of the direct pathway of the basal ganglia motor circuit by activating k-opioid receptors. In addition, proenkephalin was reduced selectively in aged PRS rats. Taken collectively, our data are consistent with an altered brain ageing in PRS rats, combined with life span impairment in the basal ganglia circuit and motor skills. This sustains the hypothesis that neurological disorders associated with pathological ageing, such as Parkinson's disease, can be unraveled starting from the adulthood, reasonably in subjects with a history of maternal stress.

Disclosures: **J. Marrocco:** None. **J. Mairesse:** None. **E. Gatta:** None. **H. Bouwalerh:** None. **M. Cannella:** None. **M. Motolese:** None. **G. Battaglia:** None. **A. Pittaluga:** None. **P. Calabresi:** None. **F. Nicoletti:** None. **S. Maccari:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.05/TT33

Topic: F.02. Animal Cognition and Behavior

Support: Grant in Aid for Scientific Research (23120008)

CREST

Title: Habenulo-raphe circuit controls adaptive avoidance behavior

Authors: ***R. AMO**[1,2], F. FREDES[1], M. KINOSHITA[1], R. AOKI[1], H. AIZAWA[1], M. AGETSUMA[1], T. AOKI[1], T. SHIRAKI[1], H. KAKINUMA[1], M. MATSUDA[3], M. YAMAZAKI[1], M. TAKAHOKO[1], S.-I. HIGASHIJIMA[4], N. MIYASAKA[1], T. KOIDE[1], Y. YABUKI[1], Y. YOSHIHARA[1], H. OKAMOTO[1,2];
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[3]Utsunomiya Univ., Utsunomiya, Tochigi, Japan; [4]Okazaki Inst. for Integrative Bioscience, NIPS, Okazaki, Aichi, Japan

Abstract: Anticipation of danger at first elicits panic in animals but later it helps them to avoid the real threat adaptively (e.g. active avoidance). The neural circuit enabling such proactive use of danger expectation is unknown. A candidate site responsible for active avoidance is the lateral habenula (LHb). In mammals, LHb neurons are suggested to have a role in transmitting anti-reward and aversive information. The Hb is conserved in vertebrates, and the zebrafish homolog of the mammalian LHb, the ventral habenula (vHb) has a simple structure including an exclusive direct projection to the serotonergic median raphe (MR). Using adult zebrafish as a model, we found that tetanus toxin mediated genetic inhibition of synaptic transmission from the vHb to the MR impaired active avoidance learning, while Pavlovian fear conditioning remained intact. *in vivo* electrophysiolosy (loose-patch recording) from genetically labeled vHb neurons during pavlovian fear conditioning showed that the vHb neuronal activity generated an expectation signal for a dangerous context. Accordingly, artificially triggering an expectation signal by optogenetic stimulation of vHb neurons in free-moving fish evoked place avoidance. We also found that optogenetic stimulation of the vHb axons in acute slice activated genetically labeled serotonergic neurons in the raphe. Thus, the vHb-MR circuit is essential for presenting danger expectation and programming active avoidance. These results reveal how perceived risk in the environment is communicated to the serotonin system to avoid potential hazard.

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Poster

748. Fear and Aversive Memories: Other Regions and Circuits

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.06/TT34

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH083702

Klarman family foundation

Title: The A2 noradrenergic circuit: a primary source of unconditioned fear

Authors: *C. M. TOURINO, S. HAYTON, R. MALENKA, L. DE LECEA;
Psychiatry, Stanford Univ., Palo Alto, CA

Abstract: Fear is an essential component of an animal's ability to anticipate harm and prepare appropriate defensive behaviors, increasing the probability of survival. However, pathological forms of fear cause phobias, panic or post-traumatic stress disorder (PTSD). There has been extensive interest in studying the generation and storage of fear memories, yet the neuronal circuits that originate unconditioned fear remain elusive. The neurotransmitter norepinephrine (NE) is central to the development of fear, but the mechanisms by which NE neurons generate a fear response are unknown. A2 noradrenergic neurons (A2 NE) in the nucleus of the solitary tract (NTS) are activated after footshock, and modulate limbic and stress-related structures. Here we show that stimulation of A2 NE neurons is sufficient to induce fear. We then demonstrate that the lateral parabrachial nucleus (lPB) mediates the majority of the responses elicited by A2 NE neurons, and that the paraventricular nucleus of the hypothalamus (PVH) redundantly controls fear-related stress responses together with the lPB, but not the physical expression of fear. This work is the first demonstration of the behavioral function of the A2 NE neuronal circuit as an efficient generator of unconditioned fear in the brain, and highlights the redundancy of fear and stress circuits, which are essential for survival.

Disclosures: C.M. Tourino: None. **S. Hayton:** None. **R. Malenka:** None. **L. de Lecea:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

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Program#/Poster#: 748.07/TT35

Topic: F.02. Animal Cognition and Behavior

Support: Undergraduate Science Education Program of HHMI

Title: 5-HT2C receptors in the bed nucleus of the stria terminalis (BNST) are implicated in the enhanced fear learning induced by SSRIs

Authors: *E. PELRINE, S. D. PASIK, E. P. BAUER;
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Abstract: Selective serotonin reuptake inhibitors (SSRIs) are a widely prescribed group of anti-depressant medications that cause increased anxiety in humans during the first few weeks of treatment. By testing the behavioral effects of acute SSRI treatment on Pavlovian fear conditioning, a well characterized model of emotional learning, we can identify how SSRIs affect the functioning of specific brain regions. A previous study revealed that administration of SSRIs given immediately prior to fear conditioning both enhances fear acquisition and increases expression of the immediate early gene Arc in both the central nucleus of the amygdala and the oval nucleus of the bed nucleus of the stria terminalis (BNSTov; Ravinder et al., 2013). This was seen after systemic injections of SSRIs as well as after direct infusion of SSRIs into the BNSTov. Here, we examine the properties of these neurons in the BNSTov which show Arc up-regulation induced by pairing an SSRI injection with fear conditioning. Rats were trained with one tone-shock pairing (5kHz, 80 dB, 30 sec tone that co-terminated with a 0.5sec 0.5mA footshock). 1 hr prior to training, animals received systemic injections of the SSRI citalopram (10mg/kg) or saline. Animals receiving SSRI injections, but not saline injections, showed Arc expression in the BNSTov; 87.2% of these neurons were also positive for 5-HT2C receptors. Next, we examined whether infusion of a 5-HT2C receptor antagonist could block the enhanced fear acquisition induced by SSRIs. 1 hr prior to fear conditioning, animals received injections of citalopram or saline and then received infusions directed at the BNSTov of either saline or the 5-HT2C receptor antagonist RS-102221 (0.5 ug/side). A long-term memory test 24 hrs later revealed enhanced freezing in the citalopram group to the tone. This increase was reduced by direct infusion of the 5-HT2C antagonist directly into the BNSTov. These data highlight the importance of the BNST in modulating fear learning and increase our understanding of serotonin's role in fear processes.

Disclosures: E. Pelrine: None. **E.P. Bauer:** None. **S.D. Pasik:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.08/TT36

Topic: F.02. Animal Cognition and Behavior

Support: R01MH065961

Title: Reversible inactivation of the bed nucleus of the stria terminalis blocks reinstatement but not renewal of extinguished fear

Authors: *T. D. GOODE[1], J. J. KIM[2], S. MAREN[1,2];
[1]Inst. for Neurosci., [2]Dept. of Psychology, Texas A&M Univ., College Station, TX

Abstract: The bed nucleus of the stria terminalis (BNST) is thought to be involved in the expression of fear to shock-associated contexts, but not to discrete conditional stimuli (CSs) paired with shock (Sullivan et al., 2004). Because context plays an important role in the extinction and relapse of fear, we sought to examine the contribution of the BNST to two different relapse phenomena: renewal and reinstatement. Male Long-Evans rats were implanted bilaterally with stainless steel guide cannulas aimed at the BNST. In the renewal experiment, rats received 5 tone CS (10 sec, 2 kHz, 80 dB)-shock unconditioned stimulus (US; 2 sec, 1 mA) trials for conditioning in "context A"; 24 hours later they received 45 tone-alone (extinction) trials in either "context B" or "context C". Ten minutes prior to a retrieval test (5 tone-alone trials), rats were bilaterally infused with either muscimol (1 μg/μl) or physiological saline at a rate of 0.3 μl/min for a total volume of 0.3 μl (per hemisphere) in the BNST. In the reinstatement experiment, rats underwent a similar procedure, but were presented with an unsignaled 'reminder' shock (1 sec, 0.4 mA) in the extinction context to reinstate fear. As before, we examined the influence of muscimol inactivation of the BNST during retrieval testing 24 hours after the reinstatement shock. In the reinstatement test, rats with muscimol infusion showed significantly less freezing than rats with vehicle infusion. In contrast, BNST inactivation did not attenuate the renewal of fear to an extinguished CS outside the extinction context. These data indicate that the BNST is involved in forms of fear relapse that depend on direct associations of the test context with the aversive US.

Disclosures: T.D. Goode: None. **J.J. Kim:** None. **S. Maren:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01MH065961 to SM

College of Liberal Arts, Texas A&M University to SM and NN

Heep Fellowship to GMA

Title: Allopregnanolone in the bed nucleus of the stria terminalis modulates sexually dimorphic contextual fear in rats

Authors: *G. M. ACCA[1], S. MAREN[1,2], N. NAGAYA[1,2];
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Abstract: In humans, sex differences in anxiety disorders are widely reported, suggesting a role for hormones and their metabolites in the regulation of fear. In rats, Pavlovian fear conditioning is sexually dimorphic; males exhibit higher levels of contextual fear compared to females. The sexually dimorphic bed nucleus of the stria terminalis (BNST) is a critical brain region for the expression of contextual fear and a potential site for its hormonal regulation. Allopregnanolone (ALLO), a progesterone metabolite, is present at higher concentrations in females compared to males, is a potent modulator of $GABA_A$ receptors (GABARs), and has anxiolytic properties, suggesting a role for this neurosteroid in the observed sex difference in conditioned fear. We hypothesized that ALLO in the BNST suppresses the expression of contextual but not cued fear in intact adult male and female rats. All rats were trained with five tone-shock pairings and tested over two subsequent days; conditioned fear was indexed by freezing behavior. On day 1, contextual fear was tested by exposure to the shock context for 10 minutes. On day 2, cued fear was tested in a novel context with four tone presentations. To determine its role in conditioned fear of males, ALLO was infused into the BNST prior to context and tone tests. In females, the role of ALLO was studied by inhibiting local synthesis and GABAR binding using separate BNST infusions of the 5-reductase inhibitor, finasteride (FIN), and the competitive neurosteroid antagonist, 17-Phenyl-(3,5)-androst-16-3n3-ol (17-PA), respectively. In males, intra-BNST ALLO significantly suppressed contextual fear. In females, intra-BNST FIN and 17-PA significantly enhanced contextual fear. None of the drugs affected cued fear in either males or females. Taken together, these results provide evidence that the anxiolytic actions of ALLO are mediated by the BNST and may contribute to sex differences in contextual fear.

Disclosures: G.M. Acca: None. **S. Maren:** None. **N. Nagaya:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.10/TT38

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1R21HD070662

Title: Corticotropin releasing factor type-1 receptor antagonism in the bed nucleus of the stria terminals impairs contextual fear conditioning

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Abstract: The function of Corticotropin Releasing Factor (CRF) as a stress-related neuropeptide in hypothalamic circuits during aversive or stressful experiences has been well characterized. However, CRF is also expressed in a limited number of extrahypothalamic limbic areas including the bed nucleus of the stria terminals (BNST) - an area where CRF is known to be important for anxiety-like behavior. Recent evidence has pointed to a pivotal role for CRF and, separately, the BNST in contextual fear learning and memory. Despite this, the function of CRF within the BNST during contextual fear learning remains poorly understood. The present study investigated the role of CRF type 1 receptors (CRFr1s) in the BNST in single-trial contextual fear conditioning and in unconditioned fear to the predator odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT). Freezing was used in both conditioned and unconditioned fear paradigms as a behavioral index of fear. We infused the selective CRFr1 antagonist antalarmin either 30 minutes prior to contextual fear conditioning (a single footshock 3 min. after being placed in a chamber) or 30 minutes prior to TMT exposure (a 10 min. long odor-only exposure session). During contextual fear conditioning, CRFr1 antagonism had no effect on post-shock freezing, but significantly disrupted freezing 24-hours later during a context-only retention test. For unconditioned fear to TMT, CRFr1 antagonism in the BNST had no effect on freezing. These data show that CRFr1 antagonism in the BNST (1) does not affect the ability to freeze when the drug is still active (i.e., intact post-shock and predator odor freezing), (2) has no effect on acquisition of contextual fear memories (i.e., intact post-shock freezing), and (3) does not affect freezing to an unconditioned fear stimulus. This suggests that CRF acting at CRFr1 receptors in the BNST may have an important function in consolidating long-term contextual fear memories.

Disclosures: A. Asok: None. **J. Schulkin:** None. **J.B. Rosen:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.11/TT39

Topic: E.05. Stress and the Brain

Support: MH73136

NS28912

MS096889

Title: Regulation of repressed hypothalamic CRH expression by augmented maternal care

Authors: *A. SINGH[1], J. MOLET[2], J. COPE[2], T. Z. BARAM[3];
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Abstract: Rationale: Augmented maternal care early in life results in a modified neuroendocrine response to stress, improved cognitive performance, and resilience to depressive symptoms later in life (Plotsky, 1993; Liu, 2000; Avishai-Eliner, 2001). Corticotropin-releasing hormone (CRH), an important component of the neuroendocrine response, is stably downregulated in the paraventricular nucleus (PVN) of the hypothalamus after augmented maternal care, which might contribute to the resulting phenotype, but the mechanisms of CRH repression are unclear. We previously found reduced number and function of glutamatergic synapses onto PVN- CRH neurons, and a stable increase of the transcriptional repressor, neuron restrictive silencing factor (NRSF) following augmented maternal care (Fenoglio, 2006; Korosi, 2010). Here, we aimed to employ controlled *in vitro* systems to examine: 1) is the decrease in glutamatergic input to CRH neurons a direct cause of downregulation of CRH mRNA and protein? 2) does NRSF contribute to this repression of CRH? Methods: Coronal hypothalamic sections containing the PVN from P6-8 rats were cultured for 6 days *in vitro*, and treated with ionotropic glutamate receptor antagonists (CNQX and MK-801) or vehicle (H2O). CRH mRNA and protein expression were determined by qRT-PCR and immunohistochemistry. Phospho-thioate modified oligonucleotides (ODNs) coding for neuron restrictive silencing element (Nrse, the NRSF consensus binding sequence) or a non-specific scrambled sequence were used to interfere with NRSF binding *in vitro*. Direct binding of NRSF to Crh gene was determined using chromatin immunoprecipitation (ChIP). Results: 1) CRH mRNA and protein were downregulated in hypothalamic slices treated with CNQX/MK-801 relative to vehicle treated controls, indicating that blocking ionotropic glutamate receptor is sufficient to repress CRH expression. 2) Co-treatment with Nrse largely reversed the CNQX/MK-801 mediated reduction in CRH. 3) CNQX/MK-801 treated samples showed increased NRSF occupancy on the Crh gene relative to vehicle treated controls. Conclusions: Repression of CRH mRNA and protein by ionotropic glutamate receptor blockade *in vitro* supports the idea that the transient decline in glutamatergic input to CRH neurons mediates downregulation of CRH following augmented maternal care. NRSF directly mediates Crh repression *in vitro*. ChIP-seq for NRSF will allow the examination of the repertoire of genes that are coordinately regulated by augmented maternal care, thus providing insight into

epigenetic effects of maternal care on cognitive and emotional phenotype of the offspring.
Support: MH73136; NS28912; MS096889

Disclosures: **A. Singh:** None. **J. Molet:** None. **J. Cope:** None. **T.Z. Baram:** None.

Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 748.12/TT40

Topic: E.05. Stress and the Brain

Support: CONICET-PIP 2065

ANPCYT/PICT 00040

Title: Intrastriatal 6-OHDA lesion in adult offspring of gestationally stressed dams

Authors: C. J. BAIER[1], M. E. PALLARES[2], E. ADROVER[2], M. R. KATUNAR[3], R. RAISMAN-VOZARI[4], *M. C. ANTONELLI[2];
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Abstract: It has been previously demonstrated in animal models, that maternal disturbances can influence the brain chemistry, endocrine function, emotionality and learning ability of the offspring. Exposure to adverse events in early life can alter adult behaviors and neurochemical indicators of midbrain dopamine (DA) activity, suggesting that the development of the DA system is sensitive to disruption by brief exposure to early stressors. In humans, dysfunctions of the dopaminergic system (DAergic) is associated with development of several neurological disorders such as Parkinson's disease (PD), schizophrenia, attention-deficit hyperactivity disorder and depression. The pathological hallmark of PD is the relatively selective loss of DAergic neurons in the substantia nigra compacta (SNc) and, to a lesser extent, in the ventral midbrain with a loss of DA contents in the striatum and the presence of cell bodies enriched in alpha-synuclein aggregates in the ventral mesencephalon. Oxidative stress and neuroinflammation participate in the pathogenesis of PD. The neurotoxin 6-hydroxydopamine

(6-OHDA) is a classical and valuable model of PD in rodents. The aim of the present study was to investigate whether prenatal restrain stress increases the vulnerability of DAergic neurons after striatal 6-OHDA injection in adulthood. We found that prenatally stressed offspring showed higher levels of cells expressing tyrosine hydroxylase (TH) in the ventral tegmental area (VTA) and that these cells were more susceptible to a neurochemical insult with 6-OHDA in adulthood. Moreover, prenatally stressed rats presented differences in terms of the number and asymmetry of neuronal nitric oxide synthase-expressing cells in the VTA and nucleus accumbens, respectively. Similar to the results described for TH-expressing cells, the nitrergic systems were differentially regulated after 6-OHDA lesion in control and prenatally stressed rats. These results indicate that prenatal stress affects both DAergic and nitrergic systems in the mesolimbic pathway. In addition, the results show that mesolimbic areas are more susceptible than motor areas to a neurotoxic insult during adult life in prenatally stressed animals.

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Poster

748. Fear and Aversive Memories: Other Regions and Circuits

Location: Halls A-C

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Topic: E.05. Stress and the Brain

Support: APIRE-Wyeth Research Fellowship

NIH Grant K08 MH086812

NARSAD Young Investigator Award

AACAP Pilot Research Award

Title: Prenatal stress induces persistent postnatal changes in both dorsal and ventral forebrain inhibitory cell populations

Authors: S. RADHAKRISHNA[1], S. LUSSIER[2], *H. E. STEVENS[3];
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Abstract: Prenatal stress has been linked to childhood behavioral and emotional disorders. GABAergic neuronal populations are implicated in these same disorders, and the prenatal development and migration of inhibitory neurons is significantly influenced by prenatal stress. In order to evaluate the postnatal development of inhibitory cell populations, CD1 GAD67GFP knock-in mice were exposed to three times daily restraint stress during the last week of gestation. BrDU injection at embryonic day 13 (E13) was used to trace the effects of prenatal stress on early born cells. Brain tissue of offspring was collected at multiple postnatal time points, and GAD67GFP+, BrdU+, and parvalbumin+ cell populations in the dorsal and ventral forebrain were assessed using immunohistochemistry and stereology. In prenatally-stressed mice at postnatal day 0 (P0), total inhibitory neuron populations were deficient in cortex and hippocampus as compared to non-stressed controls. By P24, inhibitory neuron density was increased in prenatally-stressed offspring, particularly in ventral forebrain regions. Moreover, early born GABAergic cells contributed significantly more to the greater densities in the striatum at this time point. These data provide evidence for a prenatal-stress induced migration defect in early born (E13) inhibitory cells as a contributor to the increase in cell density. By P150, medial frontal cortex and hippocampus of prenatally-stressed mice showed deficits in inhibitory neuron density while caudate putamen and amygdala continued to show higher densities of these populations. The data from P24 and P150 together suggest that postnatal processes regulating inhibitory neuron density are persistently altered after prenatal stress. Corresponding behavioral abnormalities were also observed. Adult prenatally-stressed mice showed reductions in locomotor activity correlated both with cortical inhibitory neuron deficiencies and striatal inhibitory neuron increases. Elevated plus maze testing to assess anxiety levels revealed that prenatally-stressed mice spent increased time in the closed arms, a result which correlated with inhibitory neuron densities in both the hippocampus and amygdala. This model demonstrates that both prenatal and postnatal development of GABAergic populations may be influenced by prenatal stress. Furthermore, persistent GABAergic changes in multiple brain regions may contribute to behavioral inhibition.

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Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.01/TT42

Topic: F.02. Animal Cognition and Behavior

Support: NIH 31526A

Title: Auditory imprinting in chickens: Role of PKR and thyroid hormones

Authors: *G. BATISTA[1], M. COSTA-MATTIOLI[2], J. PENA[1];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Imprinting is a form of learning that allows newly hatched chickens to recognize a particular object. Thus visual and auditory cues associated with the object must be encoded and stored for long-term recall over a short sensitive period. What mechanisms underlie these processes are still not fully understood. We imprinted chickens in laboratory settings. Immediately after hatching, we placed dark-reared chicks in a running wheel and presented 3D animated figures on a screen, coupled to a sequence of artificial sounds. Chicks preferentially approached imprinted stimuli, displaying selectivity in each sensory modality (visual and auditory) separately. We compared the difference in locomotion towards the imprinted stimulus and a novel stimulus as an indicator of imprinting strength. It has been proposed that thyroid hormones (THs) enhance visual imprinting and set the onset of its sensitive period. However, it is unknown if auditory imprinting can be also influenced by THs. We tested this possibility by injecting THs and measuring the strength of auditory imprinting. Our results show that THs injections not only enhanced visual imprinting but also auditory imprinting. Furthermore, we found that THs improved our imprinting measures by significantly decreasing approaching to novel stimuli. This raises the question of whether THs are not only affecting learning but also influencing fear responses during the sensitive period for imprinting. We injected T4 to naive chicks an measured their fear responses in a tonic immobility paradigm. Surprisingly, we found that fear responses are enhanced by T4 injections. Thus, our results suggest that THs can influence imprinting by increasing fear responses to novelty. In addition, we investigated the role of the double strained RNA-activated kinase (PKR) on the long-term memory underlying imprinting. It has been proposed that PKR is crucial for memory consolidation in rodents. PKR suppresses protein translation by phosphorylating the initiation factor eIF2α. Inhibition of PKR leads to an enhancement in long-term memory. We hypothesized that PKR may play a role in switching from short-term memory to long-term memory during imprinting. We tested this prediction by injecting chickens with a PKR inhibitor and measuring imprinting strength 3 hours and 1 day after training. As predicted, auditory imprinting was significantly better the day after training. Notably, long-term visual imprinting was not affected by this manipulation, suggesting non-overlapping mechanisms for long-term visual and auditory imprinting.

Disclosures: **G. Batista:** None. **M. Costa-Mattioli:** None. **J. Pena:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

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Program#/Poster#: 749.02/TT43

Topic: F.02. Animal Cognition and Behavior

Support: ANR MALZ

CNRS

University of Bordeaux

Title: Refining the social transmission of food preference paradigm to investigate long-lasting associative olfactory memory in rats

Authors: B. BESSIÈRES[1,2], A. GIACINTI[1,2], N. MACREZ[1,2], O. NICOLE[1,2], *B. BONTEMPI[1,2];
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Abstract: In the social transmission of food preference (STFP) paradigm developed by Galef and colleagues, rodents learn about the safety of potential food sources by sampling the odor of those sources on the breath of littermates (Galef and Wigmore, 1983, Anim. Behav. 31:748-758). We previously showed that the STFP task is particularly well-suited for the investigation of remote memory formation because 1) a single training session produces robust long-lasting memories and 2) encoding of associative olfactory memory occurs within only one brief training session which provides rigorous control over the time-course of hippocampal-cortical interactions underlying systems-level memory consolidation and avoids repeated (over days) training sessions as is often the case in complex spatial tasks (Lesburguères et al., 2011, Science, 331, 924-928). However a number of experimental factors may impact performance in this appetitive learning task. In particular, the social behavior of the “observer” and “demonstrator” rats during the interaction phase (e.g. duration of close interactions and number of nose-to-nose contacts), especially when the observer is under the influence of a drug treatment. To examine the impact of these social parameters on memory performance, we have interfaced the STFP task to a computerized video-tracking technology allowing us to automatically detect and accurately track the position of the observer and demonstrator rats during social interaction. Using an array of testing procedures and parameters (different pairs of odors, variable number or duration of interaction sessions, presence or absence of a grid to modulate the efficacy of interactions, fixed number of close interactions, various retention testing intervals), we show that the dynamics of

social exchanges between the two interacting rats is a crucial modulating factor of the initial strength and subsequent persistence of the associative olfactory memory being formed upon encoding. Thus, the amount of social olfactory information provided to observer rats during the interaction period should be carefully controlled to avoid any confounding effect on subsequent memory performance exhibited by the different experimental groups under investigation. Overall, by reducing potential confounding factors, performance variability and group sizes, our refinements enable a more standardized and reliable testing procedure for investigating remote memory formation and its functional correlates or for establishing the memory signature of various pharmacological compounds.

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Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.03/TT44

Topic: F.02. Animal Cognition and Behavior

Support: Partially supported by a Carthage College Summer Undergraduate Research Experience Grant to M. H., R. H. and P.S.

Title: Effects of acute swim stress on neutral visual object versus social olfactory recognition memory in Long-Evans rats

Authors: *P. SEYMOURE[1], M. HAYWOOD[2], R. HAMMER[2], T. TISKUS[2];
[2]Neurosci., [1]Carthage Col., KENOSHA, WI

Abstract: The contributions of separate but parallel and convergent recognition memory sensory systems can be examined using the delayed nonmatch to sample (DNMS) task (Albasser et al., 2011). The present study examined the strength of recognition memory for neutral visual stimuli and social olfactory stimuli after exposure to an acute stressor. Ten young adult male Long Evans rats briefly saw colorful objects in 4 DNMS trials (inter-trial-intervals (ITI) from 1 minute to 3 hours). A second group of 10 males had 1 minute exposures to wooden balls handled by other males in 4 Sessions with 3 trials/session. Thirty minutes prior to the last trial, 5 rats from each group were placed in a Porsolt Swim Arena for 15 minutes. Because the reliability of neutral information encoded and consolidated during acute stress has been found to be low (Qin

et al., 2012) we predicted that the forced swim would have a greater impact on hippocampal processes involved in object recognition than on the septal processes involved in odor recognition. The rats viewing neutral object stimuli spent longer investigating the novel item in Sessions 1 ($p=0.026$) and 2 ($p=0.05$), but not in Session 3 after a 3-hour ITI. There were no between group differences in inspection time across these sessions. In Session 4, after spending 15 minutes in the water, the non swimmers looked at same-same stimuli longer than the swimmers ($p=0.04$), indicating that the forced swim reduced exploration time. However forced swimming did not affect memory as both groups spent longer looking at the novel stimuli ($p=0.003$) one hour later. In the social olfactory task, the rats differentiated between balls that they had previously smelled versus a novel ball in 3 consecutive trials (1 minute ITI) per session. In Session 1 a rat smelled an own ball and a novel ball, but in the following sessions the rat examined an own ball (A), a previously smelled ball (B), and a novel ball (C). By the end of testing the rats had been presented with balls from 4 other rats. In Sessions 1-4 the rats spent significantly more time smelling the novel balls, $p=0.001$, with no difference found between the swimmers and non swimmers in time spent smelling balls after the forced swim. The findings indicate that the acute stress of forced swimming reduced examination time for neutral visual stimuli, but did not have this effect on rats that smelled balls touched by other rats. All of the rats are being tested in the Morris Water Maze task to ascertain if the forced swim has augmented or negatively impacted the spatial abilities performance of the swimmers compared to the control rats.

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Poster

749. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH086591

Title: Visual recognition in mice: perceiving the relationship between 2d pictures of objects with their 3d physical form

Authors: *S. J. COHEN[1], R. W. STACKMAN, Jr.[2];
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Abstract: Previously, our lab has demonstrated a clear and compelling role for the dorsal hippocampus in the novel object recognition task (NOR), using a number of variations on the conventional task and region-specific neuropharmacological interventions. To further assess object memory, as well as the deeper cognitive abilities of C57BL/6J male mice, a modified version of the NOR task was developed to examine the relationship that mice form between 2D pictures of real world objects with their 3D physical form. It is not clear whether rodents are capable of appropriately interpreting a pictorial stimulus as being the same as the 3D physical object it represents; a process referred to as equivalence. Reminiscent of primate and human recognition task studies, our new protocol design allows for the study of perceptual abilities in rodents. We assert that if mice spent a minimal amount of time learning about the pictures, like the imposed exploration criterion in the conventional NOR task, they would be able to correctly discriminate between these familiar pictures and either a novel picture or a novel 3D physical object. For all experiments, mice were exposed to two identical 2D pictures of either symmetrical or asymmetrical objects, during the sample session. During the test session, 24 h later, mice were required to make perceptual discriminations between 2D pictures and/or 3D objects. Memory and item perception were inferred if the mice preferentially explored the novel stimulus over the familiar, regardless of presented form (2D or 3D). For all experiments, the GABAA agonist, muscimol was bilaterally microinfused into the CA1 to temporarily block dorsal hippocampal function immediately after the sample session, while controls received saline. We were able to determine that intrahippocampal saline-treated mice are able to recall previously viewed pictures of objects, regardless of whether they were presented as 2D pictures or 3D objects during the test session. Consistent with human and primate data, the findings also confirm that mice are capable of high-level visual recognition and 2D/3D object perception are dependent on the hippocampus, extending the critical role of the hippocampus in nonspatial memory related tasks.

Disclosures: S.J. Cohen: None. **R.W. Stackman:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS 08-43175

P50 AT006268 from ODS, NCAAM, and NCI

Syracuse University

Title: A tale of two memory systems: Differential involvement in two pattern separation tasks

Authors: T. TUNUR[1], L. CASTELAN[1], W. R. HAWLEY[2], P. E. GOLD[1], *D. L. KOROL[1]; [1]Dept. of Biol., Syracuse Univ., Syracuse, NY; [2]Dept. of Psychology, Franklin & Marshall Col., Lancester, PA

Abstract: The hippocampus and striatum are two memory systems studied for their respective participation in navigation tasks that require place and response learning in water or land-based mazes. To avoid stress responses of swim tasks and food restriction in appetitive tasks, we developed two pattern separation tasks that allowed us to determine the selective involvement of hippocampus and striatum. The metric change in object location (MCOL) task measures the ability of rats to perform pattern separation using the distance between two objects. Damage to the hippocampus impairs the ability to detect MCOL (Goodrich-Hunsaker et al., 2008). The double object replacement (DOR) task examines the ability to detect the presence of two novel objects that have replaced familiar ones. Importantly, these tasks do not require extrinsic motivators or extensive locomotor activity. In the current study, we tested the role of the hippocampus or striatum in these tasks with direct microinjections of drugs that disrupt normal physiological function into these brain areas. Young adult male rats were randomly assigned to drug or vehicle control infusions and tested on either the MCOL or DOR task. Rats were allowed to explore the arena with two objects placed on opposite sides during three study sessions followed by a 30-min delay prior to the test trial. Bilateral infusions were made 10 min prior to the test trial, during which the two objects were either moved closer together for the MCOL task or replaced with new objects for the DOR task. Compared to controls, rats with disrupted hippocampal functions displayed impaired memory on the MCOL task but not on the DOR task. Conversely, rats with disrupted striatal functions displayed impaired memory on the DOR task, but not on the MCOL task. These results suggest that the MCOL task is hippocampus-sensitive while the DOR task is striatum-sensitive, demonstrating a novel dissociation between the hippocampus and striatum. These tasks will allow us to examine the metabolic and biochemical pathways involved in learning and memory without changing baseline metabolism due to stress or food/water restriction. These findings offer new behavioral methods to investigate cognitive shifts with changing hormonal states, across healthy aging, and in models of neurodegenerative diseases such as Alzheimer's or Parkinson's disease.

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Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC Grant 400176

Title: Differential contributions of de novo and maintenance DNA methyltransferases to object memory processing in the rat hippocampus and perirhinal cortex: A double dissociation due to neurogenic processes?

Authors: *K. A. MITCHNICK[1,2], S. CREIGHTON[1,2], M. O'HARA[1,2], B. E. KALISCH[3,2], B. D. WINTERS[1,2];
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Abstract: Epigenetic mechanisms are increasingly acknowledged as major players in memory formation. Specifically, DNA methylation is necessary for long-term memory (LTM) in various brain regions, including the hippocampus (HPC); however, its role in the perirhinal cortex (PRh), another structure critical for object memory, has not been characterized. Moreover, the mnemonic effects of selective DNA methyltransferase (DNMT) inhibition have not been investigated, despite their distinct *de novo* (DNMT3a, 3b) and maintenance (DNMT1) roles. Consequently, we have assessed the effects of various DNMT inhibitors (DNMTi) within the HPC and PRh of rats using the object-in-place (OiP) paradigm. The cytidine analog 5-AZA impaired long-term OiP memory when infused into the HPC, but not when administered into the PRh, whereas the non-nucleoside DNMTi RG-108 impaired long-term OiP memory in both areas. Further, intra-cranial administration of Accell siRNAs implicated DNMT3a and DNMT1 in the HPC and PRh effects, respectively. mRNA expression analyses revealed similar results, as only *de novo DNMT3a* and *DNMT3b* mRNA was upregulated in the HPC (dentate gyrus; DG) post-learning, whereas *DNMT1* mRNA was selectively upregulated in the PRh. Preliminary qPCR data suggest a heightened expression of memory-enhancing genes (*BDNF*) and a concordant down-regulation of memory-suppressing genes (*PP1*) in both areas, post-learning, while increases in neurogenesis-related genes (*Disc1, NeuroD1*) were found specifically in the DG. These results establish a functional double dissociation between the HPC/DG and PRh, demonstrating the differential involvement of DNMTs across brain regions for different types of

mnemonic processes. It is hypothesized that these differences are related to the neurogenic capacity of the HPC.

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Poster

749. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

Support: German Research Foundation - DFG (SFB936/B4)

Title: Postnatal Arc/Arg3.1 ablation causes profound impairments in long-term memory consolidation

Authors: S. CASTRO-GOMEZ, X. GAO, S. GRAF, D. KUHL, *O. OHANA;
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Abstract: Arc/Arg3.1 expression is rapidly up-regulated by acquisition of experience and by synaptic plasticity-inducing stimuli. We previously generated conventional Arc/Arg3.1 Knockout mice (constitutive KO) which constitutively lack the Arc/Arg3.1 gene. These mice show a profound impairment in the consolidation of synaptic plasticity in the hippocampus. While synaptic transmission and short-term memory was unaffected, the KO-animals have completely lost the ability to form explicit and implicit long-term memories. Because Arc/Arg3.1 expression starts early in development, Arc/Arg3.1 bears the potential to impact on brain development and thereby cause a later disturbance of long-term memory consolidation. To address this possibility we generated Arc/Arg3.1 loxP-flanked mice (Arc/Arg3.1$^{f/f}$; conditional KO) and bred those with various CaMKIIα-Cre carrying mouse lines to obtain KO mice (late KO) in which Arc/Arg3.1 is ablated in principal neurons of the forebrain during late development. For comparison we also generated KO mice (early KO) by crossing Arc/Arg3.1$^{f/f}$ mice with a CMV-Cre mouse resulting in Arc/Arg3.1 ablation during early embryogenesis in all cells including germ cells. Behavioral assessment confirms a profound impairment in the consolidation of explicit and implicit long-term memories in the early KO mice, recapitulating our previous findings in the constitutive KO. Similarly, we observed a loss of long-term memory

consolidation in the late KO mice, demonstrating that the requirement for Arc/Arg3.1 in memory consolidation is independent of any possible additional role in developmental processes.

Disclosures: **S. Castro-Gomez:** None. **X. Gao:** None. **S. Graf:** None. **D. Kuhl:** None. **O. Ohana:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.08/TT49

Topic: F.02. Animal Cognition and Behavior

Title: Revisiting the "behavioral tagging" hypothesis

Authors: ***C. M. FREIRE COBO**, C. LAO-PEREGRIN, J. U. FREY, S. FREY;
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Abstract: The 'synaptic tagging hypothesis' (STH) describes cellular mechanisms of associative interactions between heterosynaptic inputs, i.e. the interplay between local processes at populations of synapses (consisting of glutamatergic as well as neuromodulatory ones) and more distant processes, required for the formation of a memory trace. According to STH mechanisms, an early, transient form of LTP (E-LTP), induced by a weak tetanus can be consolidated into a persistent, protein synthesis-dependent late-LTP (L-LTP). The weak stimulation transiently tags synaptic inputs through local molecular changes. The application of a second strong tetanic stimulation to another input within an associative time window induces the synthesis of plasticity-related proteins (PRPs) captured by tagged synapses of the first input strengthening E-LTP to L-LTP there. Similar processes of STH were detected at the behavioral level ('behavioral tagging hypothesis' (BTH). Here, a weak learning, only able to produce short-term memory (STM) can be reinforced into long-term memory (LTM) by the exposition of the animal close in time to the weak learning with a novelty exploration that provides the PRPs. The idea is that weak learning sets transient learning tags capturing PRPs provided by novelty exploration and thus transforming STM into LTM. Only a few laboratories have shown the pairing of a relatively weak training (STM) with an event capable of inducing the synthesis of the PRPs to reinforce this STM into LTM. Therefore, we reinvestigate that in our lab. We have used two different hippocampus-dependent learning tasks that should only produce STM previously described (Moncada et al.,2011). We performed a weak inhibitory avoidance training (wIA), where rats receive a mild electrical foot shock immediately after they step down from a platform,

suppressing the step down behavior the next time they are placed on the platform. This electrical shock has to be strong enough to induce STM (tested 4 h after the shock) but not LTM (24 h). The second task used is the spatial object recognition (SOR) task. The rat is allowed to explore a familiar arena with one object, and in the test sessions (4 h - to test STM and 24 h - to test LTM) the object is switched to a new position. The rats should remember the object at 4 h but not 24 h later. We are currently defining stimulus parameters to obtain robust and reliable wIA- and SOR-STM (tag-setting). Then we will expose the animals to a novel environment (PRP-provider) during a critical time window close to the weak trainings which should result in a transformation of their STMs to LTM and will present the parameters for reliable induction of various STMs and LTMs on this regard.

Disclosures: C.M. Freire Cobo: None. **C. Lao-Peregrin:** None. **J.U. Frey:** None. **S. Frey:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.09/TT50

Topic: F.02. Animal Cognition and Behavior

Support: RO1

P 50

Title: Astrocytic mutant DISC1 and adolescent cannabis exposure synergistically interact to produce cognitive impairment in adulthood

Authors: *B. ABAZYAN, S. ABAZYAN, A. SHEVELKIN, O. MYCHKO, C. YANG, A. KAMIYA, M. PLETNIKOV;
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Abstract: Gene-environment interaction (GEI) plays a major role in the development of psychotic disorders. Astrocytes are involved in mediation of adverse effects of environmental factors. Expression of a genetic risk factor, Disrupted-In-Schizophrenia 1 (DISC1), in astrocytes allows for studying the molecular mechanisms of GEI in astrocytes. Adolescent cannabis exposure contributes to the increased risk for psychotic disorders and cognitive impairment in adulthood. Astrocytes have been found to mediate cognitive effects of tetra-cannabinol (THC), a

major component of cannabis. Thus, we evaluated the effects of chronic adolescent THC exposure on cognitive function in adult mice that express mutant DISC1 in astrocytes. Control and mutant DISC1 mice were treated with saline or THC (8.0 mg/kg) for 21 days during postnatal days (P) 32-52; and three weeks later mice were tested for novelty-induced hyperactivity in open field, anxiety in elevated plus maze, spontaneous alteration in Y maze, spatial recognition memory in Y maze, novel object recognition, place preference test and fear conditioning. Compared to all other groups, THC-treated DISC1 mice exhibited impaired spatial, object recognition and place recognition memory. These effects of TCH were dependent on expression of mutant DISC1 during adolescent exposure and were not observed in mutant DISC1 after adult THC exposure or adolescent exposure to another psychotropic drug, amphetamine. Our results suggest that astrocytic mutant DISC1 synergistically interacts with adolescent THC exposure to produce long-term cognitive impairment in adulthood. Our model is a valuable experimental system to study the molecular pathogenesis of psychotic disorders associated with marihuana abuse.

Disclosures: **B. Abazyan:** None. **S. Abazyan:** None. **A. Shevelkin:** None. **O. Mychko:** None. **C. Yang:** None. **A. Kamiya:** None. **M. Pletnikov:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

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Program#/Poster#: 749.10/TT51

Topic: F.02. Animal Cognition and Behavior

Support: Pioneer Grant 2013008915

NRF Grant 2012R1A2A2A02011838

Title: CA1 specific deletion of Cav2.1 P/Q-type calcium channel impairs spatial and contexture memories

Authors: *J. CHO[1,2], D. JUNG[1,2], H. SHIN[3];
[1]KIST, Seoul, Korea, Republic of; [2]Univ. of Sci. and Technol., Daejeon, Korea, Republic of; [3]Inst. for Basic Sci., Daejeon, Korea, Republic of

Abstract: The hippocampus expresses various types of voltage-gated calcium channels (LVGCCs) and electrophysiological and molecular mechanisms of different types in the

hippocampus have been investigated in association with behavioral functions. However, few studies have been conducted to examine the effect of Cav2.1 P/Q type channels in the hippocampus in spite of their abundant expression. There have been difficulties in measuring learning and memory ability due to severe neurological disorders such as absence seizure, abnormal respiratory activity and etc. observed in Cav2.1 null mutant. In this study, we therefore generated CA1 specific deletion of Cav2.1 KO mice (CA1-Cav2.1 KO) in order to investigate the spatial/contexture memory effects of Cav2.1 channel in CA1 region. We did not find any significant difference between the KO and control mice in the open field, spontaneous alternation and object recognition tests. This result suggests that the CA1-Cav2.1 KO mice do not have significant impairment in locomotor activity and non-spatial working memory. However, the CA1-Cav2.1 KO mice showed retardation in both water maze task and contexture fear conditioning task, which requires to learn external spatial/contexture information. Taken all, this study suggests that the deletion of Cav2.1 channels in CA1 region impairs ability to learn the spatial/context information but does not affect other spontaneous activity.

Disclosures: J. Cho: None. **D. Jung:** None. **H. Shin:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.11/TT52

Topic: F.02. Animal Cognition and Behavior

Support: Human Frontiers Science Program (Gran. No. RGY0077/2012)

Title: Systematic profiling of cannabinoid effects on memory consolidation in rats

Authors: *P. RATANO[1], M. DURANTE[1], E. YADAO[1], V. TREZZA[2], P. CAMPOLONGO*[1]; [1]Sapienza Univ. of Rome, Rome, Italy; [2]Dept. of Science, Section of Biomed. Sci. and Technologies, Univ. "Roma Tre", Rome, Italy

Abstract: Emotions have a powerful impact on memory and it is now well accepted that the most vivid memories tend to be of emotional events. Our recent studies showed that the endocannabinoid system is crucially involved in the control of emotional states and memory processes1,2. However, contrasting findings have been reported regarding the effects induced by pharmacological manipulation of the endocannabinoid signaling on cognitive functions and emotional behaviors. Several confounding variables could be responsible of the opposite data

reported in literature, among them, the different experimental context/conditions, the drug selectivity, the vehicle used to dissolve drugs, the time of administration are of crucial importance. For instance, following a pre-training administration cannabinoid drugs could strongly interfere with pain perception and/or locomotor activity at the time of training. Moreover, it is known that some endocannabinoids could also activate not only the cannabinoid receptors subtype 1 (CB1) and 2 (CB2) but also the peroxisome proliferator-activated receptors (PPARα) and the transient potential vanilloid receptors (TRPV1). Therefore, the aims of this study were a) to systematically reduce the impact of any possible confounding variables, and b) to better clarify the involvement of different signaling pathways in mediating the effect of cannabinoid compounds on memory consolidation. In order to reduce the impact of such confounding variables, we systematically employed invariable experimental conditions, drug dissolution method, time and route of administration. Thus, we administered cannabinoid compounds in order to profile the effects of these drugs on memory consolidation after post-training administration (i.p.) in rats on an inhibitory avoidance task. Thereafter, to evaluate a possible interaction between the cannabinoid compounds or endogenous cannabinoids and PPARα or TRPV1 receptors in modulating memory consolidation process, we also investigated whether the effects on memory consolidation induced by enhancing or impairing cannabinoid neurotransmission could depend on activation of PPARα or TRPV1 receptors

Disclosures: **P. Ratano:** None. **M. Durante:** None. **E. Yadao:** None. **V. Trezza:** None. **P. Campolongo*:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.12/TT53

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

CFI

Title: Overtrained contextual fear memories are resistant to hippocampal or perirhinal cortex damage, but not combined hippocampal and perirhinal cortex damage

Authors: *E. H. SHEPHERD, H. LEHMANN;
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Abstract: Context fear memories are dependent on several medial temporal lobe structures, including the hippocampus (HPC) and the perirhinal cortex (PRH). Damage to either of these structures typically causes retrograde amnesia for contextual fear conditioning in rodents. The Distributed Reinstatement Theory, however, suggests that additional learning experiences may make the memory become less vulnerable to the damage. Indeed, it has been demonstrated that overtraining in contextual fear condition mitigates the retrograde amnesic effects of complete HPC damage. The aim of this experiment was three fold: 1) replicate the finding that overtraining prevents the retrograde amnesic effects of HPC damage in contextual fear conditioning, 2) examine whether the same occurs following PRH damage, and 3) whether combined HPC+PRH damage is sufficient to cause retrograde amnesia for overtrained contextual fear conditioning. Rats were given two daily contextual fear conditioning sessions (3 min; 1 shock) distributed across 5 days. Three to five days later, the rats received sham, HPC or PRH lesions. When tested for retention, the HPC and PRH groups did not freeze significantly less than the sham group, suggesting that the memory resisted damage to either structure. Three to five days later, the same rats received a second surgery, in which the other structure was damaged, resulting in two groups: sham and HPC+PRH. When tested again for retention, freezing was significantly lower in the rats with combined HPC+PRH damage than the sham group, suggesting that the combined damage causes retrograde amnesia. Combined, these findings suggest that overtraining in contextual fear conditioning makes the memory become resistant to HPC or PRH damage. Hence, repeatedly reinstated context fear memories become independent of both the HPC and PRH, supporting the Distributed Reinstatement Theory. However, the memory does not disengage from either structure because combined damage to the HPC and PRH caused retrograde amnesia.

Disclosures: E.H. Shepherd: None. **H. Lehmann:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.13/TT54

Topic: F.02. Animal Cognition and Behavior

Title: New learning accelerates memory system consolidation

Authors: *J. HAUBRICH, L. CASSINI, A. P. CRESTANI, F. SANTANA, L. DE OLIVEIRA ALVARES, J. A. QUILLFELDT;
Federal Univ. of Rio Grande Do Sul, Porto Alegre, Brazil

Abstract: The hippocampus plays a central role in the formation, storage and expression of recent memories, but its contribution fades over time. Conversely, memory gradually becomes dependent of neocortical structures such as the anterior cingulated cortex (ACC). This reorganization process, called system consolidation, is well described but its biological purpose is a matter of debate. We hypothesized that one function of system consolidation is to maintain the hippocampus storage capacity. This assumption predicts that memory reorganization would be linked to the rate of newly stored information in the hippocampus. In other words, when substantial information is consolidated in a narrow period of time, memories would compete for hippocampus housing, leading to a faster reorganization and storage in extra-hippocampal regions. To test this hypothesis, wistar rats were trained in Contextual Fear Conditioning and tested 20 or 40 days later. Memory dependency of the hippocampus or the ACC was assessed by pre-test infusion of muscimol in these structures. A group of animals was submitted to new learning between the fear conditioning and test session, while controls remained in their home cages. New learning was induced by Morris Water Maze and Object Recognition tasks. We found that in the control group, fear memory was dependent of hippocampus and independent of ACC 20 days after training, but the opposite was observed 40 days after training. In contrast, animals that underwent new learning episodes showed hippocampal-independent and CCA-dependent fear memory 20 days after training. An additional group underwent physical activity (swimming) for the same extent of the "new learning" group and did not differ from controls. Our results show that new learning experiences can accelerate the system consolidation of a contextual fear memory, making it hippocampus-independent earlier. It suggests that a main role of memory reorganization is to preserve the hippocampal storage capacity, ensuring the possibility of new learning.

Disclosures: J. Haubrich: None. **L. Cassini:** None. **A.P. Crestani:** None. **F. Santana:** None. **L. de Oliveira Alvares:** None. **J.A. Quillfeldt:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.14/TT55

Topic: F.02. Animal Cognition and Behavior

Title: Deficits in M-current regulation lead to impaired consolidation of recognition memory in mice

Authors: *A. KOSENKO, S. MOFTAKHAR, D. L. GREENE, N. HOSHI;
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Abstract: Voltage-gated channels encoded by the KCNQ gene family conduct the M-type potassium current. The M-current is classically suppressed by muscarinic m1 receptor activation through a mechanism that requires phosphorylation of the KCNQ2 subunit. The M-current is one of the key modulators of synaptic plasticity with a proposed role in memory processing. It facilitates hippocampal "exploratory" theta resonance (2 - 7 Hz) critical for temporal coding at depolarized potentials. On the other hand, inhibition of the M-current leads to a temporary release of subcortical tonic inhibition allowing memory coding. In the present studies we aimed to identify the effects of M-current inhibition on memory processes. Our lab has generated knock-in mice that carry an alanine mutation at the key phosphorylation site of KCNQ2, KCNQ2(S559A). These mice show attenuated response to muscarinic-mediated M-current suppression, enabling us to address the role of physiological M-current suppression in memory processing. To discriminate between the memory processes mediated by different brain regions, we conducted perirhinal cortex-dependent Novel Object Recognition (NOR) and hippocampus-dependent Novel Location Recognition (NLR) tasks. KCNQ2(S559A) mice showed normal long-term spatial memory as evidenced by successful performance on the NLR task with a 24 h retention interval. However, we observed a significant long-term recognition memory impairment in KCNQ2(S559A) mice compared to the wild-type on the NOR task with a 24 h retention interval. Inhibition of the M-current with XE991 rescued long-term recognition memory in KCNQ2(S559A) mice when administered 15 min before or immediately after the training session. This suggests that M-current suppression plays a role in consolidation of recognition memory. KCNQ2(S559A) mice also showed deficits in long-term social odor memory, while maintaining normal olfactory responses, further implicating the M-current in memory processes mediated by perirhinal cortex. Finally, our behavioral findings were mirrored by a lower level of neuronal activation in perirhinal cortex of KCNQ2(S559A) mice compared to the wild-type during memory consolidation, as measured by c-fos expression 2 h after NOR training. Our findings provide evidence for the proposed importance of M-current suppression during memory processing and offer a novel perspective on its role in recognition memory consolidation.

Disclosures: A. Kosenko: None. **S. Moftakhar:** None. **D.L. Greene:** None. **N. Hoshi:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: TOP Grant, Dutch Organization for Scientific Research (NWO)

Title: Memory retrieval-extinction procedure increases spontaneous recovery of nicotine seeking, but not spontaneous recovery of cocaine seeking

Authors: R. F. STRUIK, J. DEDONCKER, Y. MOURIK, J. PETERS, *T. J. DE VRIES; NCA VU & Vumc, Amsterdam, Netherlands

Abstract: Introduction: Smoking is a major preventable health risk worldwide. An important aspect of smoking maintenance is relapse, which can occur irrespective of the time spent in abstinence. Animals will reliably self-administer nicotine, the main psychoactive ingredient of cigarettes. Repeated exposure to contingent cues (analogous to cue exposure therapy in humans) will reduce drug seeking over time, but if this therapy is discontinued, drug-seeking can re-merge, termed spontaneous recovery. In a memory retrieval-extinction procedure, the timing of the cue exposure sessions is manipulated to produce a lasting reduction in relapse propensity for both cocaine and heroin, in both rats and humans (Xue et al., 2012). The experiments in the current study aim to reduce spontaneous recovery of nicotine seeking through such a memory retrieval-extinction procedure. Materials and Methods: In experiment 1, male Wistar rats were trained to self-administer i.v. nicotine (40 µg/kg/infusion) paired with a light/sound cue (1 hour/day, for 16 days, up to a fixed ratio 4; FR4). Following self-administration training, the retrieval-extinction group was exposed to 10 minutes of contingent cue exposure (FR4), followed by a 10-min delay and a 60-min extinction session. The no-retrieval group was exposed to a single 70-min extinction session. After 16 days of the retrieval-extinction procedures and 34 days of abstinence, spontaneous recovery was measured. In experiment 2, the memory retrieval-extinction paradigm described in experiment 1 was applied to rats self-administering cocaine (500 µg/kg/infusion). Based on the results of the first two experiments, two additional experiments were performed during which rats were trained on FR1 schedules only. An FR4 schedule includes a number of unrewarded responses during self-administration training, retrieval and extinction, which may already have formed an extinction memory. Results: Nicotine trained rats, irrespective of training schedule (FR1 or FR4) showed a higher number of responses for nicotine cues during spontaneous recovery in the retrieval-extinction group compared to the no-retrieval group. Spontaneous recovery of cocaine seeking was not affected by the retrieval-extinction procedure. These findings are in sharp contrast to what is described in literature, indicating the importance of further study before the memory retrieval-extinction

procedure is applied to humans, and in particular smokers, since in this study the procedure enhanced nicotine seeking in rats.

Disclosures: R.F. Struik: None. **J. DeDoncker:** None. **J. Peters:** None. **T.J. De Vries:** None. **Y. Mourik:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.16/TT57

Topic: F.02. Animal Cognition and Behavior

Support: FAPERGS

CNPq

Title: Reactivation in the presence of distractor stimulus disrupt memory reconsolidation in rats

Authors: *A. P. CRESTANI, J. HAUBRICH, F. Z. BOOS, R. O. SIERRA, F. SANTANA, J. M. DURAN, Q. K. ZANONA, F. D. DUTRA, L. DE OLIVEIRA ALVARES, J. A. QUILLFELDT;
Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil

Abstract: A newly formed memory becomes a stable memory trace through a consolidation process that follows acquisition. However, memory reactivation can initiate a new transiente labile phase, named reconsolidation, through which it can be enhanced, impaired or updated. Thus, reconsolidation provides an opportunity to change unwanted fear memories. Furthermore, psychotherapies that distract the patient during traumatic memory retrieval were shown somewhat effective. In this study, we used adult male Wistar rats trained in the contextual fear conditioning task. We hypothesized that a distractor stimulus during memory reactivation could interfere with fear memory reconsolidation in rats previously conditioned. Our results demonstrate that memory reactivation in the presence of distractor stimulus was able to impair the expression of the original fear memory during reactivation session. When animals reexposed (memory reactivated) in the presence of distractor were newly exposed in the context previously associated with footshocks they continued showing long-lasting fear attenuation and in a later test any spontaneous recovery was observed. We also found that the disruption of original memory depends on the destabilization-reconsolidation process: when animals were exposed to a

novel context memory destabilization did not occur and distractor stimulus failed to disrupt aversive memory. Moreover, drugs that prevent memory destabilization, such as the L-VGCC antagonist (i.p.) and GluN2B antagonist (intrahippocampal), infused before the reactivation session, prevented fear memory attenuation. Thus, it was possible to interfere with a previously acquired fear memory by inhibiting memory reconsolidation through distraction, a finding that may have relevant clinical implications for the treatment of emotional disorders, once it can be an animal model for understanding the mechanisms that underlie the distraction psychotherapies strategies may allow therapy improvements.

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Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.17/TT58

Topic: F.02. Animal Cognition and Behavior

Title: A novel UCS-retrieval extinction procedure prevents relapse to drug seeking

Authors: ***Y. LUO**[1], M. JIAN[1], Y. HAN[1], Y.-X. XUE[1], J.-F. LIU[1], Y. SHAHAM[2], L. LU[1]; [1]Natl. Inst. On Drug Dependence , Peking Univ., Beijing, China; [2]Natl. Inst. on Drug Abuse, Natl. Inst. of Hlth., Baltimore, MD 21224, USA, MD

Abstract: Background: We recently reported that a memory-retrieval-extinction procedure decreases reinstatement of drug seeking in rats and drug craving in humans; drug-related memories were retrieved by exposure to drug-associated conditioned stimuli (CSs) (Xue et al. Science 2012). Here we determined whether the inhibitory effect of the memory-retrieval-extinction procedure on relapse also occurs when memory retrieval is induced by exposure to the self-administered drug (the unconditioned stimulus [UCS]). Methods: We trained rats to self-administer cocaine for 10 days (3h/d). We then injected them with saline or cocaine (3 mg/kg) 1 h or 9 h prior to daily extinction sessions. Next, we tested for cocaine seeking induced by drug-priming injections (reinstatement), passage of time (spontaneous recovery), or re-exposure to the drug context (renewal). Results: Non-contingent cocaine injections (UCS retrieval) 1 h but not 9 h prior to the extinction sessions decreased reinstatement, spontaneous recovery, and renewal of drug seeking. The UCS retrieval manipulation also delayed reacquisition of cocaine self-

administration and decreased neuronal activity in prefrontal cortex, nucleus accumbens and amygdala. Unlike the CS-retrieval-extinction manipulation, the UCS-retrieval-extinction manipulation inhibited drug seeking induced by discrete drug-associated cues not present during extinction training and also inhibited drug seeking when the manipulation started after 28 abstinence days. Blockade of the UCS-induced AMPAR endocytosis in BLA abolished the inhibitory effect of UCS-extinction procedure on cocaine-priming-induced reinstatement. Conclusions: The UCS memory retrieval-extinction procedure has "superior" relapse prevention characteristics than the CS memory retrieval-extinction procedure and could be a promising method for decreasing craving and relapse in human addicts.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH 5R01MH084038

Title: Out-of-context activation memory: Temporal gradient of memory enhancement

Authors: *B. B. LEE[1], A. A. FENTON[2];
[1]Physiol. & Pharmacol., SUNY Downstate Med. Ctr., BROOKLYN, NY; [2]Ctr. for Neural Sci., New York Univ., New York City, NY

Abstract: Background: We previously showed that a stressful experience can modify unrelated memories such that its subsequent expression is enhanced (Ježek et al, PLoS Biol 2010). A 20-min forced-swim induces a stress response that raises serum corticosterone levels to 300% above baseline, and the swim environment had no physical, hormonal, or other identifiable feature in common with the learning context. Rationale: The lingering consolidation hypothesis proposes that an acquired memory progressively undergoes consolidation with time such that the stability of the memory gradually increases and becomes more resilient to disruption in a kind of temporal gradient. The working hypothesis is that the stability of a memory increases in a temporal gradient such that swim stress can no longer modify that memory. We investigated whether there is a temporal gradient within which swim stress can enhance the expression of an

unrelated memory. Methods: After training in an aversively-conditioned left/right discrimination task in a T-maze, rats were subjected to either a 20-min forced-swim or non-swim conditions 1, 7, 14, or 30 days later. Retention was then tested 1 day later. Since the training establishes a robust memory, retention was measured by switching the safe/shock arms, and measuring the number of error trials. Results and Summary: Swim stress enhanced the expression of memories that were 1-week-old or younger, as compared to that of non-swim controls. However, swim stress had no impact on memories that were 2-weeks-old and older. Also, the left/right discrimination training regimen produced a robust memory that persisted a month after learning as 20/26 (77%) animals initially chose the arm that they had learned (test of proportions, one-tail $z = 2.75$; $p < 0.003$). These data suggest that there is a temporal gradient within which swim stress can enhance unrelated memories, and that the window closes at some point between 1 week and 2 weeks.

Disclosures: B.B. Lee: None. **A.A. Fenton:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

Support: Caledonian research fellowship (Royal Society of Edinburgh)

Royal Society (London) research grant

Title: Novel events facilitate persistence of contextual memory through consolidation and reconsolidation

Authors: *S.-H. WANG;
Ctr. for Clin. Brain Sci., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Novel events occur around the time of memory encoding can facilitate the memory persistence of trivial details that otherwise fades away (Wang et al, 2010, Salvetti et al, 2014). This phenomenon, observed in rats, mimics the flashbulb memories experienced in humans. The molecular mechanisms underlying this facilitation of long-term memory are highly similar to those observed in the persistence of long-term potentiation in hippocampal slices. Together, the facilitated persistence of memory or synaptic potentiation can be explained by the synaptic

tagging and capture theory (Redondo and Morris, 2011). However, it remains unclear whether the novel events can also facilitate memory persistence though memory recall and reconsolidation. Hence, this study aimed to answer first, whether novel events can enable memory to last longer when they are present at the time of memory recall, and second, whether the novel events can enable the memory to persist when the memory reconsolidation is inhibited. I used an appetitive 1-trial place learning paradigm as well as contextual fear conditioning to investigate this. The results showed that strong appetitive place encoding on day 1 led to good memory recall on day 2 which faded away on day 3. When a novel event was introduced before or after the memory recall on day 2, the memory could then last till day 3. Furthermore, in a contextual fear conditioning paradigm that led to good memory recall and reconsolidation, the reconsolidation could be impaired by beta-adrenergic blocker treatment and such impairment could be rescued by a novel event introduced at memory recall. Together, the evidence supports that novel events can facilitate memory persistence at reconsolidation and suggest a tagging and capture-like mechanism at memory recall. Redondo R and Morris RGM (2011) Nat Rev Neurosci 12:17-30. Salvetti B, Morris RGM, Wang SH (2014) Learning and Memory 21:61-72. Wang SH, Redondo R, Morris RGM (2010) PNAS 107:19537-42.

Disclosures: S. Wang: None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.20/TT61

Topic: F.02. Animal Cognition and Behavior

Title: Is selective hippocampal cholinergic deafferentation sufficient to produce temporally graded retrograde amnesia?

Authors: J. R. KÖPPEN[1], S. S. STUEBING[1], M. SIEG[1], A. A. BLACKWELL[1], P. BLANKENSHIP[1], E. D. GRISLEY[2], J. L. CHEATWOOD[2], *D. G. WALLACE[1];
[1]Northern Illinois Univ., DEKALB, IL; [2]Southern Illinois Univ. Sch. of Med., Carbondale, IL

Abstract: Dementia of the Alzheimer's type (DAT) is a neurodegenerative disorder marked by degeneration of basal forebrain structures and is associated with significant mnemonic deficits. The current study used a rat string-pulling task to evaluate whether selective cholinergic deafferentation of the hippocampus is sufficient to produce temporally graded retrograde amnesia. Female rats were pre-trained to pull strings to obtain reinforcement (cashew).

Subsequently, rats were trained to discriminate between two scented strings. One scented string was consistently reinforced (+A), while the other scented string was never reinforced (B). After rats met criterion, they either waited two weeks (recent) or six weeks (remote) prior to receiving a sham surgery or infusion of 192-IgG-Saporin into the medial septum. Two weeks later rats were given four days of reversal training during which they experienced the same scented strings; however, the cashew was at the end of the string that was not previously reinforced. Following reversal training, rats were trained on a novel discrimination (+C/D). The results of the current study are consistent with selective cholinergic deafferentation of the hippocampus being sufficient to produce retrograde amnesia that was not temporally graded. First, all rats met criterion in a similar number of days. Rats receiving infusion of 192-IgG-Saporin into the medial septum had a higher number of correct responses during reversal training, relative to sham rats; however, no group differences were observed between recent and remote groups. Next, there were no group differences in the ability to learn a new discrimination. Finally, no group differences we observed in the latency to approach and pull up the string. The results were not caused by deficits in motivation or motor function, but they do reflect impairments in mnemonic function. The current study provides a novel behavioral assessment technique that models the retrograde amnesia characteristics observed in DAT.

Disclosures: J.R. Köppen: None. **D.G. Wallace:** None. **S.S. Stuebing:** None. **M. Sieg:** None. **A.A. Blackwell:** None. **P. Blankenship:** None. **E.D. Grisley:** None. **J.L. Cheatwood:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: MRC Grant G0700991

Leverhulme Trust Grant F/00 094/BK

Title: Reconsolidation of memories underlying instrumental responding

Authors: M. T. EXTON-MCGUINNESS, R. C. PATTON, L. B. SACCO, *J. L. LEE; Univ. Birmingham, Birmingham, United Kingdom

Abstract: Following their retrieval, many memories have been demonstrated to destabilize, necessitating a process of memory reconsolidation to restabilize the memory and ensure its persistence. This cycle of destabilization and reconsolidation is thought to mediate the updating of memories. It is notable, therefore, that there have been no published reports of the memories underlying instrumental behaviour undergoing reconsolidation. Indeed, several studies have failed to demonstrate the existence of instrumental memory reconsolidation, either in direct investigations, or in studies of the pavlovian modulation of instrumental responding. Here, we have revisited instrumental memory reconsolidation, focussing on the hypothesised link between reconsolidation and memory updating. We trained male rats to acquire an instrumental lever press response for sucrose reward under continuous reinforcement. Rats were trained for either 2 or 10 days. They were then subjected to a variety of reactivation sessions, having been injected with MK-801 (0.1 mg/kg, i.p.) or saline. The integrity of the underlying instrumental memory was subsequently tested in extinction. Following 2 days of training, neither a brief extinction session nor a brief instrumental training session caused the instrumental memory to destabilize. However, exposure to a change in reinforcement contingency to a variable ratio 5 schedule, designed to engage memory updating mechanisms, rendered the memory vulnerable to the amnestic effect of MK-801 to impair responding at test in a reactivation-dependent manner. Following 10 days of training, an extinction session was again ineffective in triggering reconsolidation. Instead a VR20, but not VR5 or FR20, session successfully destabilized the instrumental memory, allowing MK-801 to disrupt its reconsolidation and reduce instrumental responding at test. Together, these results demonstrate that instrumental memories do undergo reconsolidation under specific conditions that correspond with memory updating. Weak instrumental training results in instrumental responding that is sensitive to reward devaluation, and so it seems likely that goal-directed action-outcome instrumental memories do reconsolidate. It remains unclear, however, whether the performance deficit at test after the stronger training reflects an impairment in action-outcome and/or habitual stimulus-response instrumental memories. Nevertheless, the ability to disrupt the reconsolidation of well-learned instrumental memories, resulting in diminished instrumental performance, has potential therapeutic applications for addictive drug seeking behaviours.

Disclosures: **M.T. Exton-McGuinness:** None. **R.C. Patton:** None. **L.B. Sacco:** None. **J.L. Lee:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.22/TT63

Topic: F.02. Animal Cognition and Behavior

Title: Insulin-like growth factor-II affects on memory consolidation and persistence with restricted time window

Authors: *S. PARK, Y. LEE, Y. LEE, Q. GAO, Y. LEE, E. KIM, H. LEE, J. RYU;
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Abstract: Memory consolidation is one of the important phases at memory formation. Recently, it was reported that insulin-like growth factor-II (IGF-II) enhances memory consolidation and prevents memory forgetting. Previously, we reported that mature brain-derived neurotrophic factor enhances memory consolidation with restricted time window, within 9 h after the acquisition trial. Thus, we hypothesized that IGF-II also exerts its activity on memory consolidation with restricted time window. In the present study, hippocampal injection of IGF-II at 6 and 9 h after the acquisition trial significantly increased step-through latencies compared with the vehicle-treated controls at 24 hours post-training, whereas, at 12 h after the acquisition, exogenous IGF-II could not increase step-through latencies. On the other hand, hippocampal injection of IGF-II at 6, 9 and 12 h after the acquisition trial significantly increased step-through latencies compared with the vehicle-treated controls at 21 days post-training. The effect of exogenous IGF-II at 9 h after the acquisition trial on memory consolidation and the effect of exogenous IGF-II at 12 h after the acquisition trial on memory persistence were both abolished by co-injected anti-IGF-II receptor antibody. These results suggest that IGF-II has restricted or effective time window on the enhancement of memory consolidation, within 9 h after acquisition trial and also, a delayed consolidation phase, 12 h after the acquisition trial is critical for memory persistence, but not formation.

Disclosures: S. Park: None. **Y. Lee:** None. **Y. Lee:** None. **Q. Gao:** None. **Y. Lee:** None. **E. Kim:** None. **H. Lee:** None. **J. Ryu:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: CAPES

IBRO

CNPq

UFRGS

Title: HDAC inhibition form a precise contextual memory and keep its accuracy over time

Authors: *L. O. ALVARES[1], F. D. DUTRA[2], L. PEDRAZA[2], A. P. CRESTANI[2], R. O. SIERRA[2], J. A. QUILLFELDT[2];
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Abstract: Although many memories fade after a time-point, becoming more schematic, some old memories seem to retain their original precision. Animal studies have reported a gradual increase in generalization of contextual fear conditioning over time (i.e. a diminishing ability to distinguish a context associated with a footshock from a novel context. Memory consolidation requires de novo gene expression, in which is regulated by epigenetic machinery, including histone acetylation. Recent studies have shown significant increases of histone acetylation levels after memory acquisition in the hippocampus. In the present study, we examined the role of histone deacetylase (HDAC) inhibitor Sodium butyrate (NaB) during initial learning in memory precision both in recent and remote memory. We first show that NaB increase histone H3K9-14 acetylation levels following contextual fear conditioning in the hippocampus. Next, we evaluated if animals treated with NaB would express a higher discrimination ratio between a context previously associated with a footshock and a novel one. Two days after training, both groups were able to distinguish both contexts in a "standard protocol". Then, we challenged animals to discriminate between two contexts very similar. NaB treated animals, but not the control group, were able to dissociate both contexts, expressing higher freezing levels in the training context. To address if NaB would prevent the loss of content specificity over time, animals were trained in contextual fear and tested 40 days later in the conditioning or the novel context. NaB treated group expressed higher freezing levels in the training context than in the novel context, whereas the control group froze equally in both contexts. This data strongly support that the histone acetylation levels during consolidation play a critical role in making a contextual rich memory. Thus inhibiting HDAC both strengths memory and maintains its accurate/precise representation over time.

Disclosures: L.O. Alvares: None. **F.D. Dutra:** None. **L. Pedraza:** None. **A.P. Crestani:** None. **R.O. Sierra:** None. **J.A. Quillfeldt:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.24/TT65

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

Title: Systemic Gastrin-Releasing Peptide attenuates reconsolidation of contextual fear memory in rats

Authors: *A. MURKAR[1], C. CAYER[2], P. KENT[2], J. JAMES[2], Z. MERALI[2];
[1]Univ. of Ottawa, Gatineau, QC, Canada; [2]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Post traumatic stress disorder (PTSD) is characterized by an inability to extinguish fear memories. Research suggests gastrin releasing peptide (GRP), a bombesin-like neuropeptide found in mammals, may mediate processes of fear learning. The GRP receptor is highly expressed in the lateral nucleus of the amygdala (a key region implicated in the formation of fear memories), specifically on inhibitory GABAergic interneurons. Central administration of GRP reduces the expression of learned fear. Recent findings suggest that reactivated memories must be reconsolidated following recall if they are to be maintained; this provides a window of opportunity where pharmacological manipulation may prevent those memories from being successfully reconsolidated for long-term storage. The present study aimed to assess the effects of systemic GRP injection on the reconsolidation of conditioned fear memories. Using contextual fear conditioning in male Sprague-Dawley rats, the effects of GRP (10 nmol dose, administered I.P. immediately following memory reactivation) were assessed. The results demonstrated that systemic GRP administration following memory reactivation significantly attenuated reconsolidation of the learned fear response. In addition, co-administration of Flumazenil (a GABAA receptor antagonist) blocks this effect, suggesting that the ability of GRP to interfere with memory reconsolidation is through a GABAergic mechanism. These findings suggest that GRP may offer a novel treatment avenue for disorders such as PTSD.

Disclosures: **A. Murkar:** None. **P. Kent:** None. **C. Cayer:** None. **J. James:** None. **Z. Merali:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS 080073

McLean Hospital

Title: Xenon gas impairs reconsolidation of fear memories in a rat model of PTSD

Authors: M. J. KAUFMAN, *S. E. LUKAS, T. E. GILLIS, J. MANOUKIAN, E. G. MELONI; McLean Hosp., BELMONT, MA

Abstract: Xenon (Xe) is a noble gas that has been developed for use in people as an inhalational anesthestic and a diagnostic imaging agent. Xe inhibits glutamatergic N-methyl-D-aspartate (NMDA) receptors involved in learning and memory and can affect synaptic plasticity in the amygdala and hippocampus, two brain areas known to play a role in fear conditioning models of post-traumatic stress disorder (PTSD). Because glutamate receptors also have been shown to play a role in fear memory reconsolidation - a state where recalled memories become susceptible to modification - we examined whether Xe administered after fear memory reactivation could affect the expression of subsequent fear-like behavior (freezing) in rats. Naïve male Sprague-Dawley rats were trained for contextual and cued fear conditioning; rats received two pairings of a 30 s, 75 dB tone co-terminating with a 0.6 mA footshock. Twenty-four hours later, freezing to the conditioning context and conditioned stimulus (tone) was measured. Immediately after this test, which served to reactivate the fear memory, rats were exposed to inhaled Xe (25%) or normal room air and the effects on fear memory reconsolidation were measured 48 and 96 hr after reactivation/Xe administration. A second set of animals was trained as described above but did not receive a reactivation test 24 h later. Instead, at this time-point, animals were exposed to 25% Xe for 1 h to determine whether Xe must be paired with memory reactivation for it to affect memory reconsolidation. A third set of animals was trained as described above, underwent reactivation 24 hours later, and were exposed to Xe (25%, I hr) beginning 2 h after the reactivation test, to determine whether delayed Xe exposure affected freezing 48 and 96 h later. A fourth set of animals was trained as described above and exposed to Xe (25%) for 1 h twice to determine whether multiple Xe exposures enhance reconsolidation blockade. Xe administration immediately after fear memory reactivation - but not when given 2 hr after reactivation -

significantly reduced freezing to the context and cue when tested 48 and 96 hr after reactivation/Xe administration. In contrast, Xe administration in the absence of fear memory reactivation had no effect on subsequent freezing behavior. Multiple Xe exposures did not further enhance the reconsolidation blockade. These data suggest that xenon inhibits memory reconsolidation in a reactivation and time-dependent manner, that it could be used as a new research tool to characterize reconsolidation and other memory processes, and that it could be developed to treat people with emotional memory disorders such as PTSD.

Disclosures: M.J. Kaufman: None. **S.E. Lukas:** None. **E.G. Meloni:** None. **T.E. Gillis:** None. **J. Manoukian:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 749.26/TT67

Topic: F.02. Animal Cognition and Behavior

Title: Protein kinase M zeta and the maintenance of hippocampal–dependent long–term memory

Authors: *D. C. GIDYK[1], S. H. DEIBEL[2], R. J. SUTHERLAND[2];
[1]Neurosci., Univ. of Lethbridge, Lethbrigde, AB, Canada; [2]Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: How memories persist in the brain remains a fundamental question in neuroscience. One aspect of this question is: how memories can be maintained at the level of the synapse, despite constant molecular turnover (Crick, 1984). Currently, most of what is known about the molecular mechanisms of memory is restricted to the induction of memory, not memory maintenance. Within the last 20 years, protein kinase M zeta (PKMζ) has emerged as a prime candidate that may be involved in the molecular mechanism of long-term memory (LTM) maintenance. Experimental evidence suggests that inhibition of PKMζ can cause erasure of certain types of memory (for a recent reviews, see Sacktor, 2011; Glanzman, 2013; Kwapis and Helmsetter, 2013). However, there is a lack of compelling evidence linking PKMζ to the maintenance of hippocampal LTM. Most of the studies that have shown retrograde amnesia after the inhibition of PKMζ investigated memories stored in neocortical networks. In the studies that have found effects of the inhibition of PKMζ on HPC-dependent memory, the deficits were minor, or the results unclear (Serrano, 2008). Specifically, Serrano et al suggest that the inhibition of PKMζ in HPC with ZIP may have disrupted details of LTM, but not course

information. It then follows that PKMζ may not be required for the maintenance of all types of LTM, but is somehow involved in the mechanism. Another consideration is the method in which PKMζ is inhibited in the vast majority of the aforementioned studies. Zeta inhibitory peptide (ZIP) is thought to act as a psuedosubstrate, specifically binding to PKMζ and rendering it inactive (Hernandez 2002). However, there is evidence that strongly suggests ZIP may not be specific to PKMζ and there is some doubt whether ZIP inhibits PKMζ at all (Volk, 2013). The present study was designed to further investigate the role of PKMζ in the maintenance of two HPC−dependent memories via its inhibition with ZIP or a second method of transient blockage. The results of this study will provide insight into the role of PKMζ in HPC−dependent LTM, address the specificity of ZIP, and establish an alternative for the transient inhibition of PKMζ *in vivo*.

Disclosures: D.C. Gidyk: None. **S.H. Deibel:** None. **R.J. Sutherland:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant GM-69337

Title: Specific details of auditory memory and cortical plasticity are regulated by hdac3

Authors: *K. BECHAY[1], K. BIESZCZAD[2], M. WOOD[2];
[1]Univ. of California- Irvine, Fullerton, CA; [2]Univ. of California- Irvine, Irvine, CA

Abstract: Understanding the molecular mechanisms underlying complex brain functions, such as the storage and recall of long term memory, will have critical implications on the way we view and treat neural disease or decline. Histone acetylation, an epigenetic mechanism that promotes a transcriptionally active DNA conformation, has been recently implicated in the enhancement of brain plasticity and several types of memory. Presently, we demonstrate that histone acetylation is also critical for sensory specificity in cortical, associative memories- which has never been demonstrated before. Rats were injected with an HDAC3 inhibitor, thus promoting histone acetylation, immediately after an auditory task in which they associate a specific frequency with reward. These rats, compared to vehicle-injected control rats, have stronger, more specific memories to the learned frequency as defined by behavioral tests. Also,

electrophysiological recordings show that the representation of that learned frequency in the primary auditory cortex (A1) was increased beyond the controls, demonstrating enhanced cortical plasticity with HDAC3-inhibition. HDAC3-inhibited animals also learned an additional frequency that the control animals did not, suggesting that HDAC3 may regulate the threshold of memory induction. It is clear from these results that HDAC3 is a critical, negative regulator of cortical memories and we hope that this initiates further investigation into the pathway to HDAC3 regulation, as well as its downstream effects.

Disclosures: K. Bechay: None. **M. Wood:** None. **K. Bieszczad:** None.

Poster

749. Memory Consolidation and Reconsolidation: Behavior

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC Discovery Grant

Alexander Graham Bell NSERC CGS

Title: Adolescent immune system stimulation with the immunogen lipopolysaccharide (LPS) alters the acute-phase response and lithium chloride (LiCl)- induced conditioned place avoidance acquisition following a homotypic challenge in adulthood in the female rat

Authors: *C. J. CLOUTIER, M. KAVALIERS, K.-P. OSSENKOPP;
Dept. of Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: The bacterial endotoxin, lipopolysaccharide (LPS), has been shown to impair learning and memory in a number of paradigms, such as, rapid gustatory conditioning and the acquisition of conditioned disgust reactions (i.e., gaping) in the rodent model of anticipatory nausea. LPS has also been shown to produce no effect on learning (i.e., Morris water maze). Furthermore, immune system challenge at the critical developmental period of adolescence and its effects on future immune challenge outcomes in adulthood is not well-studied. This study examined the effects of immune system stimulation with LPS, in adolescence and/or adulthood, on the acquisition of toxin (LiCl)-induced conditioned place avoidance (CPA). At 42 days old (adolescent), 64 female Long Evans rats were intraperitoneally (ip) injected with either LPS (200 μg/kg), or 0.9% isotonic saline (NaCl; 1 ml/kg). At 60 days old (adult), subjects underwent a

CPA procedure (2 cycles of 4 consecutive days, spaced 72 h apart). On drug-paired conditioning days (days 1,3,7,9), rats were treated with systemic (intraperitoneal) LPS or NaCl 90 minutes prior to a second injection of either 0.15M lithium chloride (LiCl; 15 ml/kg) or NaCl (15 ml/kg), immediately followed by a 30 minute exposure to a specific context (gray wall, rough floor) and limited to the right chamber. On control days (days 2,4,8,10), rats were treated with NaCl 90 minutes prior to a second injection NaCl, immediately followed by a 30 minute exposure to a different context (striped wall, wire floor) and limited to the left chamber. On a 20 minute drug-free test day, on which subjects were permitted to explore either of the two chambers, rats pre-treated with LPS 90 minutes prior to LiCl conditioning spent significantly more time in the drug-paired chamber relative to NaCl pre-treated rats that were conditioned with LiCl, showing that LPS impaired the acquisition of CPA. However, treatment with LPS in both adolescence and adulthood produced tolerance to the deleterious effects of LPS on learning and memory, evidenced by a significantly shorter period of time spent in the drug-paired chamber that was comparable to other LiCl-treated animals not treated with LPS. Furthermore, following the first conditioning day, LPS-treated rats that were also treated with LPS in adolescence exhibited a tolerance-like effect to the acute-phase response sickness behaviours, lethargy and anorexia. In conclusion, this study shows that a single systemic immune system challenge with LPS at 6 weeks (adolescence) produces long-term alterations in the acute-phase response and central learning/memory impairments that have previously been shown to follow immune system stimulation.

Disclosures: C.J. Cloutier: None. **M. Kavaliers:** None. **K. Ossenkopp:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Topic: F.02. Animal Cognition and Behavior

Support: Mercator Stiftung

Title: Functional evidence for a distal CA1- proximal subiculum- lateral entorhinal cortex network in recognition memory

Authors: *N. H. NAKAMURA, M. M. SAUVAGE;
Mercator Res. Group 1 (FAM), Fac. of Med., Ruhr Univ. Bochum, Bochum, Germany

Abstract: It has been suggested that the lateral entorhinal cortex (LEC) and the distal part of CA1 (close to the subiculum) process preferentially nonspatial information. LEC principally projects to distal CA1 and nonspatial information is believed to flow from the superficial layer of LEC to the hippocampus and back to the deep layer of LEC. This feedback is thought to either be direct or to involve the proximal part of the subiculum, which receives preferentially distal CA1 inputs. However, functional evidence for this network is sparse and whether it is sensitive to memory demands remains elusive. To address these issues, we employed Arc imaging method to visualize neuronal activation in the deep and superficial layers of LEC, the proximal subiculum and distal CA1 during the retrieval of odor recognition memory. Rats performed a delayed non-matching-to-sample task with high or low memory demands (5-odor vs. 10-odor recognition; the total number of items per day and the number of training sessions was equated between groups). We found that the deep layer of LEC, proximal subiculum and distal CA1 were sensitive to memory demands (e.g. were more activated in rats from the 10 odors group than in rats from the 5 odors group). In contrast, this was not the case for the superficial layer of LEC. Hence, these results suggest the existence of a functional network between distal CA1 and the deep layer of LEC, which could also involve proximal subiculum, and demonstrate its involvement in the retrieval of memory.

Disclosures: **N.H. Nakamura:** None. **M.M. Sauvage:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NRF Grant WCI 2009-003

Title: Representation of objects in the CA1 region of the hippocampus

Authors: ***T. GEILLER**[1,3], D. JUNG[2], J.-S. CHOI[3], S. ROYER[2];
[1]Ctr. for Functional Connectomics, [2]KIST, Seoul, Korea, Republic of; [3]Korea Univ., Seoul, Korea, Republic of

Abstract: The hippocampus plays a critical role in episodic memory, which is believed to require an integration of object and place information. While a number of studies outline the impact of spatial factors and recent history on place cells activity, little is known on how the

identity of objects might be represented in the hippocampus. To investigate this question, we trained mice to run for water rewards on a long treadmill belt on which small objects could be attached or removed at will. Typically 3 types of objects with contrasting color and texture were used: 1) a 'forest' of ~3 cm long spines made with hot glue, 2) a 'floor' of scattered shrink tubes, and 3) a 'floor' of scattered pieces of Velcro. Each object spanned over 10 cm of the belt, and was repeated at 2 to 4 locations of the belt. Importantly, the objects' positions were attributed randomly to avoid any repeating sequence of objects. A ~5ul sweet water reward was delivered through a lick port at a specific belt position at every trial. After 3 weeks of training, two 64-channels silicon probes held in stereotaxic manipulators were inserted (under isoflurane anesthesia) into the CA1 and CA3 regions of the hippocampus. Recordings started ~30-60 min after insertion, when the mice fully recovered from anesthesia and started running. A total of 20 recording sessions (CA1: 19 sessions, CA3: 8 sessions) were performed in a total of 9 mice. Two types of cells were noticeable in the CA1 regions: Cells that exhibit a single place field on the belt, akin to typical 'place cells', and less expected, cells which exhibit firing fields tightly coupled to a specific type of object, which we refer for convenience as 'object cells'. Object cells firing activity was restricted to the vicinity of a specific object and was repeated in a similar fashion at all the positions of the object. Moreover, the object fields disappeared or appeared instantly when the objects were removed or added to the belt, indicating that the fields depend mostly on a detection mechanism rather than on learning and memory of objects location. Adding unfamiliar objects created instant object fields as well, indicating that object representation did not require prior knowledge of the objects. In contrast to CA1, no object cells could be observed in CA3, which exhibited mostly broadly tuned single place fields. This finding might be consistent with higher integration of place and object information in the CA3 recurrent network. Our results show another difference between CA3 and CA1 representations, with objects identity being represented by a subpopulation of CA1 hippocampal cells.

Disclosures: **T. Geiller:** None. **D. Jung:** None. **J. Choi:** None. **S. Royer:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Program#/Poster#: 750.03/TT72

Topic: F.02. Animal Cognition and Behavior

Title: Effects of hippocampal administration of AMPA receptor antagonist NBQX on the retrieval of spontaneous object recognition memory

Authors: *E. TAKANO, K. YAMADA, Y. ICHITANI;
Univ. of Tsukuba, Ibaraki-Ken, Japan

Abstract: Research on the role of hippocampus (HPC) in object recognition memory has produced conflicting results. Previous studies using hippocampal lesions suggest the possibility that HPC is important for object recognition memory when the delay between the sample and test phases is long enough. Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors are one of the main excitatory neurotransmitter receptors. In this study, we examined the role of hippocampal AMPA receptors in the retrieval of short-term and long-term (6 hr, 24 hr, 72 hr, 1 wk and 3 wk) spontaneous object recognition memory by using 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide(NBQX)-disodium, an AMPA receptor antagonist. Male Wistar-Imamichi rats, 8-9 wk old at the beginning of the experiment, were used. Rats were allowed to explore a pair of identical objects (A) in an open-field arena (90×90×45 cm) for 5 min repeatedly (24 hr - 3 wk groups) or once (6 h group) in the sample phase. After the delay, novel object preference was assessed by comparing the amount time spent exploring the familiar (A) versus novel (B) object in the test phase. Exploration of an object was defined as directing the nose to the object at a distance of <2 cm and/or touching it with the nose. The time spent exploring the novel and familiar objects was recorded for 5 min, and the first 2 min was analyzed. We calculated the discrimination ratio (DR), the time spent exploring the novel object divided by the total time spent exploring both objects. Rats received bilateral hippocampal injection of either phosphate buffere (PB) or NBQX (5.0 mM, 1 μL) 15 min before the test phase. For the microinjection, rats were implanted with guide cannulae in the dorsal HPC 1 wk before the test phase (3-wk delay group) or 1 wk before the sample phase (the other groups). Under PB treatment, rats showed significantly higher level of DRs compared with chance level (50%) in all delay groups (71 - 78%). However, under NBQX treatment, DRs in all groups were not significantly different from chance level (6 hr: 44%, 24 hr: 60%, 72 hr: 52%, 1 wk: 57%, 3 wk: 52%). These results suggest that dorsal HPC plays an important role in the retrieval process of spontaneous object recognition memory even the delay between the sample and the test phases is short enough as 6 hr.

Disclosures: E. Takano: None. **K. Yamada:** None. **Y. Ichitani:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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PRONEM 003/2011 FAPERN/CNPq

PRONEM 12/2010 FACEPE/CNPq

Title: Unique spike avalanches emerge during exploration of novel objects in rats

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Abstract: Neuronal avalanches measured as consecutive bouts of thresholded field potentials are conceived to be a statistical signature that the brain operates near a critical point. In theory, criticality optimizes stimulus sensitivity, information transmission, computational capability, and mnemonic repertoire size. Another interesting aspect regarding field potential avalanches, recorded via multielectrode arrays from cortical slice cultures, is that they are diverse and yet precise spatiotemporal activity patterns, which repeat for many hours. It remains unclear whether spike avalanches, characterized by uninterrupted activity over consecutive temporal bins and observed in forebrain regions of freely-behaving rats, also form recursive repertoires, and whether these have any behavioral relevance. Here we show that spike avalanches recorded from the hippocampus as well as from the sensory neocortex of freely-behaving rats, in addition to providing evidence of criticality in the brain *in vivo*, also constitute distinct families of recursive spatiotemporal patterns. A significant number of the patterns identified were specific to waking, whereas avalanches occurring during sleep were typically shared by waking repertoires. More importantly, the exposure to novel objects increased both the rate at which new avalanche families arose, and the diversity of post-exposure repertoires. Although a sizeable proportion of spike avalanche families occurred specifically during periods of whisker contact with the objects, very few were strongly associated to specific objects. We conclude that spike avalanches, beside establishing criticality as a feature of the brain dynamics, also conform repertoires that emerge in waking, recur during sleep, are diversified by novelty and may contribute to the representation of objects.

Disclosures: **T.L. Ribeiro:** None. **S. Ribeiro:** None. **M. Copelli:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MHO86591

Title: Object-specific activity recorded from the hippocampus of male C57BL/6J mice and a novel behavioral paradigm to assess discrimination of moving 3D objects

Authors: *H. N. ASGEIRSDOTTIR, R. W. STACKMAN;
Psychology, Florida Atlantic Univ., Jupiter, FL

Abstract: The cognitive map theory states that the hippocampus creates representations of locations where relevant non-spatial items or objects are encountered and where specific events occur within a contextual or spatial reference frame. The rodent hippocampus is an essential neural substrate for spatial memory and the CA1 region has been shown to play a vital role in object memory dependent and independent of context; findings consistent with the cognitive map view. *In vivo* extracellular recordings from the distal band of dorsal CA1 in freely moving C57BL/6J mice, yielded recordings of both place cells and cells that demonstrated object-specific activity over consecutive sessions with objects present. Object-specific activity was observed when the mouse explored novel and familiar objects, but was independent of spatial location where objects were found, or object identity. The firing frequency of these object-specific cells increases over time as mice explore the objects and become familiar with them in different locations. These results raise questions regarding 3D objects in motion and how they are processed within the rodent hippocampus. We developed a novel behavioral protocol to test the influence of motion on object memory. The "Knowing your enemy" task requires mice learn to discriminate between two moving objects and avoid proximity to only one of them to prevent receiving a foot shock. The task requires the mice to both discriminate the objects and continuously update their location in space, both of which are believed to be hippocampal-dependent. Temporary inactivation of the hippocampus in mice completing the "knowing your enemy" should result in altered avoidance of the correct object. These results further support the involvement of the rodent hippocampus in non-spatial object memory.

Disclosures: H.N. Asgeirsdottir: None. **R.W. Stackman:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NC3Rs Grant NC/K500252/1

Title: Investigating recognition memory using immediate-early gene imaging in the rat brain

Authors: *K. E. AMEEN-ALI[1], M. J. EACOTT[1], J. A. AINGE[2], B.-A. ROBERTSON[1], A. EASTON[1];
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Abstract: Many studies have demonstrated the importance of the perirhinal cortex in detecting object novelty but there is still debate about its functional links with the hippocampus. The spontaneous object recognition procedure is often used to assess animals' memory of previously encountered objects through normal spontaneous exploration of novel objects. This procedure can be used with immediate-early gene (IEG) imaging to investigate the expression of fos protein in the rat brain during recognition memory. With the standard one-trial procedure, however, it can be difficult to assess recognition memory with IEG imaging as c-fos activity is normally most readily quantifiable after many trials. The continual trials apparatus provides an alternative testing method to the one-trial approach as it allows for animals to be tested for multiple trials within a session with no need to handle the animals between trials. The present study investigated the expression of c-fos when three groups of Lister hooded rats were tested on a object recognition task using the continual trials apparatus. All groups were tested on the same set of objects but Group Familiar had prior exposure to these objects; Group Novel had prior exposure to a different set of objects; Group Naïve had no prior exposure to objects. All groups displayed significant recognition in the task. Exposure to familiar stimuli prior to the object recognition test was associated with lower fos expression in the perirhinal cortex. Object novelty in the object recognition test was associated with increases in fos expression in the perirhinal cortex and variable c-fos patterns in the hippocampal subfields. These findings support previous evidence which suggests that fos expression in the rat perirhinal cortex increases after viewing novel visual stimuli compared to viewing familiar visual stimuli. Applying the multiple trial recognition procedure to IEG imaging means multiple brain regions can be imaged simultaneously allowing us to answer key questions about network interactions in the medial temporal lobe when rodents explore novel and familiar objects. The present findings provide

further support for the role of the perirhinal cortex in detecting object novelty but also potentially implicate hippocampal involvement. Further work will investigate c-fos activation during recognition of familiar object-location and object-context configurations.

Disclosures: K.E. Ameen-Ali: None. **M.J. Eacott:** None. **J.A. Ainge:** None. **B. Robertson:** None. **A. Easton:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Shota Rustaveli National Science Foundation grant of Doctoral DO/203/7-240/13

Title: Age dependent effect of toluene chronic exposure on recognition memory

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Abstract: The present study has been undertaken to determine whether toluene chronic exposure provokes immediate and/or persisting effect on exploratory behavior and recognition memory in open field in adolescent and adult rats. Reaction to novelty is a behavior frequently observed in mammals when they are confronted with the changes in their physical environment. The open field gives the opportunity to reveal any alteration in such behavior. Normal mammals are able to detect a new object added to familiar ones. To the change of location of one or more familiar object/s that have been set up in an open field, they usually react by renewed exploration of the entire apparatus and/or by reinvestigation of the displaced object. Using the same behavioral tests, we investigated possible effect of a single toluene inhalation. Single exposure had no effect on exploratory behavior and recognition memory. Therefore, alterations described in the present study should represent the consequence of toluene chronic (and not single) exposure. We exposed male Wistar rats at ages P 28-32 (adolescents) and P 70-75 (adults) to 2000 ppm inhaled toluene for 40 days. The immediate and persisting effects of toluene misuse (immediately after the end of toluene chronic inhalation and 90-day after the end of toluene chronic inhalation, correspondingly) were evaluated. The major findings are: (1) toluene misuse alters exploratory

activity and recognition memory in adolescent and adult rats; (2) the level of alterations depends upon the postnatal age of testing animals. In particular: in adolescent rats the most significant behavioral alterations were observed by the day following toluene chronic exposure. These alterations do not progress significantly during abstinence period: some altered parameters were almost the same as observed the day following immediately after toluene misuse and others were very close to observed in control animals. Therefore, in adolescent rats the most expressed was immediate effect of toluene misuse. Contrary to it: in adult rats most alterations significantly progress during 90 d period of abstinence. So, in these animals more substantial was persistent effect of toluene chronic exposure. On the bases of our data it is possible to suggest that adolescent rats may show partial recovery from once the toluene toxic effect no longer persists.

Disclosures: **N. Pochkhidze:** None. **M. Zhvania:** None. **N. Japaridze:** None. **M. Dashniani:** None. **M. Burjanadze:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Maternal immune activation produces deficits in a displaced object recognition task

Authors: J. G. MILLAR, *D. K. BILKEY;
Psychology, Univ. of Otago, Dunedin, New Zealand

Abstract: Maternal immune activation (MIA) is a risk factor for schizophrenia. In animal models MIA offspring display behavioural, cognitive and neurophysiological deficits, including alterations in hippocampal function and long-range EEG coherence. Here we tested the effect of MIA on the hippocampal-dependent, displaced object recognition memory task while also recording EEG from the dorsal hippocampus (dHPC) and prelimbic medial prefrontal cortex (mPFC). Pregnant rat dams were injected with polyinosinic-polycytidilic acid (Poly I:C) or saline on GD15 and their adult male offspring served as subjects. For five recording sessions animals were exposed to two identical objects, always positioned in the same location. For the sixth session, conducted 3 hours later, one of the objects was displaced to a new location. Control animals spent longer investigating the displaced object, whereas MIA animals distributed their exploration evenly between the two objects (group difference in discrimination ratio; p=.002), indicating a dysfunction in spatial recognition memory in MIA animals. Analysis

of the EEG indicated that there was no difference in theta-frequency magnitude coherence between dHPC and mPFC, either between groups or in the EEG generated while animals explored the two objects. There was however, a significant increase in theta-frequency phase coherence between these two regions as animals shifted from a no-exploration to exploration of the familiar, and then the displaced, object ($p=.008$). There was also a significant positive relationship between the behavioural bias that animals showed towards exploration of the displaced object and an increase in mPFC theta amplitude that occurred as animals shifted their exploration from the familiar to the displaced object ($r=0.67$, $p<0.01$). This exploration-related change in mPFC theta amplitude differed between the MIA and control groups ($p=0.05$). Whereas amplitude increased as the control animals shifted exploration from the familiar to displaced object, in the MIA group the opposite pattern occurred. In summary, the MIA intervention altered exploratory behaviour towards a displaced object, mirroring the deficits in spatial memory observed in individuals with schizophrenia. This effect was not reflected in between-group alterations in EEG coherence, however, some MIA-associated changes in EEG properties, in particular, the response of the mPFC to exploration was observed. These data show that prenatal immune challenge alters spatial recognition memory in the offspring and provides some indication as to how the dHPC and mPFC may communicate during this process.

Disclosures: J.G. Millar: None. **D.K. Bilkey:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Title: Hippocampal lesions do not affect measures of recollection and familiarity in rhesus monkeys

Authors: *B. M. BASILE[1], R. R. HAMPTON[2];
[1]NIMH/NIH, Bethesda, MD; [2]Emory Univ., Atlanta, GA

Abstract: One influential model of recognition posits two underlying memory processes: recollection, which is detailed but relatively slow, and familiarity, which is quick but lacks detail. Whether the hippocampus is necessary for both recollection and familiarity, or only for recollection, has been a topic of much debate. Previous work with nonhuman primates has shown that hippocampal lesions sometimes do not affect overall recognition accuracy. It is possible that this is because the hippocampus only supports recollection, and monkeys with hippocampal damage primarily use a familiarity-based strategy. To distinguish between recognition based primarily on familiarity and recognition based on a combination of familiarity and recollection, we analyzed recognition errors as a function of response latency. In twelve normal monkeys (Macaca mulatta), choices were made in three different ways depending on response latency. Quick errors were disproportionately false alarms to familiar lures, consistent with control by vague familiarity. Medium-latency responses showed fewer false alarms and higher accuracy, consistent with the onset of a recollective process that could correctly reject familiar lures. Slow responses were guesses, indicated by low accuracy and equal rates of false alarms and misses. A response deadline selectively increased false alarms, suggesting that limiting processing time weakened the contribution of recollection and strengthened the contribution of familiarity. Together, these findings suggest that monkey recognition performance is consistent with a dual-process model consisting of quick familiarity and slower recollection. To test the competing hypotheses about hippocampal contributions to recognition, we induced selective excitotoxic hippocampal lesions bilaterally in five monkeys and retained five as controls. Although initial post-lesion testing suggested a minor group difference, the effect was transitory. Both groups continued to show high false alarm rates after short latencies and a reduction in false alarms with medium latencies. To further evaluate whether hippocampal damage increased reliance on item familiarity, we varied the familiarity of the unstudied lures both within and between sessions, and the baseline familiarity of test stimuli. In all cases, control monkeys made as many false alarms as did monkeys with hippocampal damage. The current data suggest that the monkey hippocampus is not critical for item recognition. To the extent that this paradigm measures both recollection and familiarity, hippocampal damage does not induce a recognition strategy that is disproportionately based on familiarity.

Disclosures: B.M. Basile: None. **R.R. Hampton:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Topic: F.02. Animal Cognition and Behavior

Support: DFG Schw 559/12-1

Title: Hippocampal lesions boost performance in the rat sequential reaction time task: Evidence that enhanced instrumental experience is not critical

Authors: S. BUSSE[1], *R. K. SCHWARTING[2];
[1]Behavioral Neurosci., [2]Philipps-University of Marburg, Marburg, Germany

Abstract: It is commonly accepted that the hippocampus plays a major role in declarative memory across various species, for example, spatial memory in rodents. However, the interplay between hippocampal function and nondeclarative learning and memory, like procedural stimulus-response (S-R) or sequential learning, is less clear. Recently (Eckart et al. Hippocampus 22'12, 1202-1214), we showed that excitotoxic lesions of the rat dorsal hippocampus led not only to the expected deficits in a spatial and presumably declarative task, that is, the object-place recognition test, but also to substantial improvements in terms of both, speed and accuracy in a rodent adaption of the human serial reaction time task (SRT), that is, a task where performance is usually attributed to striatal mechanisms (Eckart et al. Neurotox. Res.17'10, 487-298). This effect was subsequently replicated by us in a rat model of mesial temporal lobe epilepsy with classic hippocampal sclerosis (Will et al. Behav. Brain Res. 247'13, 65-72). The designs of both experiments, however, which included fixed test durations per training day, led to the fact that lesioned animals gained more instrumental experience, since they were substantially faster and more accurate than controls. This factor may partly have accounted for their enhanced performance. In order to rule out such a potential confound, we performed the present experiment on rats with ibotenic lesions of the dorsal hippocampus, where we kept the amount of correct instrumental responses and reinforcement on the same level as in controls. Nevertheless, rats with dorsal hippocampal lesions clearly outperformed controls in the SRT, indicating that the higher levels of instrumental experience, which rats with similar lesions had in our previous experiments, did not substantially account for the lesion-induced boost of performance in the rodent SRT. In line with our previous lesion findings, these data support the hypothesis that loss or impairment of hippocampal function can enhance specific task performance, especially when it is dependent on procedural (probably striatum-dependent) mechanisms with minimal spatial requirements.

Disclosures: S. Busse: None. **R.K. Schwarting:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Title: Noradrenergic and dopaminergic hippocampal activity encodes environmental novelty information

Authors: D. AVILA-AGUIRRE, *F. BERMUDEZ-RATTONI, P. MORENO-CASTILLA; UNAM, Mexico City DF 04510, Mexico

Abstract: It has been suggested that recognition memory has two components; a sense of familiarity with the features of a particular stimulus and recollection of the stimulus in the context where it was experienced. The dorsal hippocampus receives high-level inputs that include context information. So it is in our interest to understand the mechanisms by which the hippocampus process contextual matching information. We performed *in vivo* microdyalisis in order to monitor extracellular changes in neurotransmitters levels during Object Location Memory (OLM), a behavioral protocol developed for evaluation of contextual information in recognition memory. Neurotransmitter release was evaluated in dorsal hippocampus and insular cortex during OLM in 3 to 4-month-old B6129SF2/J mice. Additionally, we administered 6-hydroxydopamine in the dorsal hippocampus in a different set of mice. We found a release of dopamine and norepinephrine in hippocampus during OLM, while neurochemical activity remained unaltered in cortex. Depletion of catecholaminergic terminals in hippocampus by 6-OH dopamine lesion impaired OLM. Our results support the relevance of hippocampal catecholaminergic neurotransmission in memory, despite the role of catecholamines in emotional arousal. Even more the significance of catecholaminergic function may be extended to the clinical field as it has been reported that innervation of hippocampus by noradrenergic system is reduced and atrophied in non-pathological aging and Alzheimer's disease patients.

Disclosures: **D. Avila-Aguirre:** None. **F. Bermudez-Rattoni:** None. **P. Moreno-Castilla:** None.

Poster

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Support: Spanish BFU2011-29089

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Title: Involvement of hippocampal and prefrontal circuits in unpredictable situations and in novelty detection

Authors: ***A. GRUART**[1], I. FERNÁNDEZ-LAMO[2], R. SÁNCHEZ-CAMPUSANO[2], J. DELGADO-GARCÍA[2];
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Abstract: The role of cortical and subcortical circuits in associative learning tasks is well established. Usually, these tasks are presented to the experimental animal in a rather standardized way, including a repetition of equally-designed training sessions. Nevertheless, real life situations involve the presence of unpredictable changes in cues or contexts, introducing additional difficulties for the appropriate acquisition of the selected task. Thus, it would be important to understand the involvement of hippocampal and prefrontal circuits in unpredictable situations and/or in novelty detection. Rats were divided in four groups and implanted with: i) bipolar stimulating electrodes in the perforant pathway (PP) and recording electrodes in the ipsilateral hippocampal CA1 area; ii) stimulating electrodes in the CA1 area and recording electrodes in the ipsilateral medial prefrontal cortex (mPFC) and in the subiculum (SUB); iii) stimulating electrodes in the mPFC and recording electrodes in the ipsilateral nucleus accumbens (NAC) and the basolateral amygdala (BLA); and iv) electrodes to record simultaneous activity in these five sites. Implanted electrodes were used to record activity-dependent changes in synaptic strength and local field potentials (LFP) evoked during selected behaviors. Rats were trained in Skinner boxes with a fixed-ratio (FR1:1) schedule. Once trained, animals were transferred to a modified box with two infrared photoelectric sensors to detect animals approaches to the lever. In selected random trials, the lever was removed when the animal's head reached the first photoelectric sensor (at 10 cm away from the lever). Synaptic activation or LFP recordings were

carried out when the animal crossed the second photoelectric sensor (at 2 cm from the lever). Preliminary results indicate that CA1-mPFC, mPFC-NAC, and mPFC-BLA synapses increase the amplitude and/or slope of evoked fEPSPs during the acquisition of the instrumental task, but this increase in strength was significantly reduced *in situ*ations of unpredictability, i.e., when the animal approached the lever without a previous information of its availability. Baseline controls for each fEPSP were collected with the animal placed in a small box, 2 min before the test. In contrast, PP-CA1 synapses were not modified in any of these two experimental situations, whilst the CA1-SUB synapse was depressed in the two situations. Spectral analysis of recorded LFPs indicate the presence of significant changes in power and in coherence (mostly between mPFC and NAC, and between CA1 and SUB) during control (lever present) and unpredictable (lever absent) situations.

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Poster

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J. Edward and Helen M.C. Stern Endowed Professorship in Neuroscience

Title: A shortened version of the object-in-place visual recognition paradigm detects spatial and object recognition memory in juvenile C75BL/6J mice

Authors: M. FLORES-MONTOYA[1], J. ALVAREZ[3], *C. SOBIN[2];
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Abstract: The object-in-place visual recognition paradigm effectively measures spatial and object recognition memory in mice. As previously described (De Viti et al., 2010), the paradigm

required 60 minutes per animal which can reduce feasibility for studies using large numbers of animals. Previously published studies have not used this test for assessing spatial and object memory in juvenile animals. The present study describes results from a 40 minute version of the paradigm conducted with juvenile mice. C57BL/6J mice from three litters (N = 19, 11 males and 8 females) were tested at PND 28 (pre-adolescence). Animals were habituated to the empty 80 cm diameter circular testing arena on PND 25, 26 and 27 (10 min per session). On PND 28, animals were tested in 7 consecutive sessions (S) of 4 min duration, with a 2 min interval between sessions. For S1, animals were placed in the arena without objects. For S2, S3 and S4 animals were exposed to 5 objects placed in upper, lower, left, right and center areas of the arena. For S5 and S6 the locations of two familiar objects were changed. For S7, one novel object was substituted for one familiar object in a familiar location. Data regarding the animals movements were collected via a camera mounted over the arena and analyzed using the SMART software system (Harvard PanLab). The main outcome variable, total percentage of exploration time was analyzed with two-way ANOVA comparing familiar and novel exploration time, controlling for litter. Mice spent significantly more time exploring objects in a novel as compared with familiar location (S5, $F = 16.82$, $p < .01$; S6, $F = 9.67$, $p < .01$), and more time exploring the novel as compared with familiar objects (S7, $F = 21.57$, $p < .01$). The findings demonstrated that the shorter 40 minute version of the object-in-place paradigm adequately detected spatial and object recognition memory in juvenile C57BL/6J mice. For studies using more than a small number of animals, this shortened paradigm offers significant time savings, improved feasibility and reduced animal fatigue. Importantly, the paradigm detected memory effects in pre-adolescent (PND 28) C57BL/6J mice. Spatial and object recognition memory have been linked to dentate gyrus pathway function, thus this task could be especially useful for conditions that alter dentate gyrus, for example, as has been observed in young low-level lead exposed mice.

Disclosures: **M. Flores-Montoya:** None. **C. Sobin:** None. **J. Alvarez:** None.

Poster

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The University of Delaware Undergraduate Research Program

Title: Neonatal ethanol exposure on postnatal days 7-9 disrupts object-in-place learning and trace fear conditioning in juvenile rats

Authors: *L. E. BRENNAN, M. E. STANTON;
Psychology, Univ. of Delaware, Newark, DE

Abstract: The purpose of this study was to extend our previous work on neonatal alcohol exposure and object recognition performance (Jablonski et al., 2013) to the object-in-place (OiP) task. This task requires memory of both object identity and object location, and therefore serves as a combination of the standard object-recognition (OR) and object-location (OL) tasks (Barker et al., 2007; Barker & Warburton, 2011; Jablonski et al., 2013). The present study utilized 4-Object and 2-Object variants of the OiP task that were modeled after other studies (Barker et al., 2007; Ainge & Langston, 2012, respectively). Rats preferentially explore novelty--in the OiP task, an object is novel if it changed locations with another object (4-Obj) or replaced another object (2-Obj) between sample and test phases. In the 4-Object variant, 4 different objects are presented during the sample phase; the locations of the 2 left or right objects are interchanged for the test phase. The 2-Object variant consists of 2 different objects during the sample phase; one of these objects is replaced with an identical copy of the remaining object for the test phase. Lesion studies have implicated roles of prefrontal cortex (PFC), hippocampus (HPC), and perirhinal cortex (PRH) in this task (Barker studies, cited above), while trace fear conditioning (TFC) is believed to engage both PFC (Gilmartin & Helmstetter, 2010) and HPC (McEchron et al., 1998). Additionally, neonatal alcohol disrupts maturation of PFC (Whitcher & Klintsova, 2008) and HPC (Marino et al., 2004). Therefore, we predicted that neonatal alcohol would disrupt OiP learning, as well as visual TFC as a "positive control" task (Schreiber et al., 2012). We report that normative PD26 rats can perform the 4-Object, but not 2-Object, variant of the OiP task, which may imply reliance of these tasks on different neural mechanisms. Neonatal ethanol exposure during postnatal days (PD) 7-9 disrupted both the 4-Object variant of OiP in PD26 rats, as well as TFC--but not background contextual conditioning during TFC--in PD30-31 rats. These findings underscore the previously supported claim that OR, OL, and OiP tasks rely on different neural regions and/or systems; furthermore, they are interesting in the context of our recent report that PD7-9 ethanol exposure does not impair OR and OL tasks (Jablonski et al., 2013). Future studies could better inform understanding of the relationship between ethanol exposure window, brain targeting, and behavioral deficits.

Disclosures: L.E. Brennan: None. **M.E. Stanton:** None.

Poster

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University of Delaware Undergraduate Research Program

Title: Differential ontogeny of object-in-context and object-place-context memory in the rat

Authors: *A. I. RAMSARAN, M. E. STANTON;
Psychology, Univ. of Delaware, Newark, DE

Abstract: The novelty-preference paradigm is comprised of a wide variety of tasks that assess different processes of incidental object, spatial, contextual, and temporal learning and memory. Recent literature has shown that, in adult rats, variants of this paradigm rely on different neural systems (e.g., Barker et al., 2007), which makes these tasks particularly useful for investigating neurocognitive development. The present study focused on two of these task variants that have been associated with hippocampal function (Mumby et al., 2002; Langston & Wood, 2010). The object-in-context (OiC) task (Dix & Aggleton, 1999) measures associative object-context memory, as evidenced by preferential exploration of an object in an incongruent context, over an object familiar to that context, during the test phase. Alternatively, the object-place-context (OPC) task (Eacott & Norman, 2004) is a model of "episodic-like memory" because it assesses memory for an object, the context in which it appeared, and its spatial location within the context. Performance of the OPC task is indicated by heightened exploration of an object in a novel configuration of location and context, over an object in a familiar location and context, during the test phase. In comparing the ontogenetic profiles of the OiC and OPC tasks in Long-Evans rats, we found that rats as young as PD17 could perform the OiC task, while PD31 rats, but not PD26 rats, could perform the OPC task. Additionally, through the application of a novel control condition, we observed that novelty preference in all groups was abolished when rats were tested in an alternate (yet, familiar) context not experienced during the study phase. These results suggest that episodic-like memory develops later in ontogeny compared to associative object-context memory and that memory in both of these tasks is mediated by the association of objects identities or object locations (OiC and OPC tasks, respectively) to context representations during the study phase.

Disclosures: A.I. Ramsaran: None. **M.E. Stanton:** None.

Poster

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Support: R21-HD070662-01

Title: Neonatal alcohol exposure impairs incidental spatial learning in the juvenile rat: Effects of exposure scenario

Authors: *S. R. WESTBROOK, M. E. STANTON;
Psychology, Univ. of Delaware, Newark, DE

Abstract: Fetal alcohol spectrum disorders (FASDs) are neurocognitive disorders that include behavioral impairments resulting from exposure to alcohol during gestation. Early exposure to alcohol leads to deficits in spatial memory (Dokovna, Jablonski, & Stanton, 2013; Jablonski & Stanton, 2014; Goodlett & Johnson, 1997; Goodlett & Pearson, 1995; Murawski & Stanton, 2010, 2011). Using the object location recognition task, incidental (non-reinforced) spatial learning was examined in a rat model of FASDs in which rats received binge-like (5.25 g/kg/day) alcohol exposure during the third trimester-equivalent or sham-intubations. In Experiment 1, rats exposed to alcohol or sham-intubated from postnatal day (PD) 7-9 and tested on PD26 performed the object location task by preferentially exploring the object in the novel location compared to the object in the familiar location after both 5-min and 24-hr retention intervals. In Experiment 2, rats exposed to alcohol from PD4-9 and tested on PD26 did not preferentially explore the displaced object, while the sham-intubated rats did show a preference for the displaced object. The presence of an alcohol deficit in juvenile rats following PD4-9 exposure but not PD7-9 exposure suggests that the different exposure windows may target different brain areas and/or processes underlying incidental spatial learning tasks.

Disclosures: S.R. Westbrook: None. **M.E. Stanton:** None.

Poster

750. Animal Models: Recognition Memory and Novelty Detection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 750.17/TT86

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH038752

Title: Constitutively active glycogen synthase kinase-3 impairs cognitive task performance in mice

Authors: *M. PARDO, R. S. JOPE;
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Abstract: Brain GSK3 is hyperactive in several conditions associated with impaired cognition, such as Fragile X syndrome (FX) and Alzheimer Disease. This raises the possibility that uncontrolled activity of GSK3 contributes to impaired learning and memory. Therefore, the purpose of this study was to test if hyperactive GSK3 in the mouse brain is sufficient to impair cognition. GSK3 is predominantly regulated by inhibitory phosphorylation on GSK3α on serine-21 and GSK3β on serine-9. Therefore, we assessed cognition in GSKI3 knockin (KI) mice with serine-to-alanine mutations in serine-9 of GSK3β and serine-21 of GSK3α which maintain GSK3 maximally active but at physiological levels, as it is not over-expressed. Cognition was assessed in these mice compared to matched wild-type (WT) mice in four tasks that previously were found to be impaired in FX mice: novel object recognition, temporal order memory, coordinate spatial memory, and categorical spatial memory task. Whereas WT mice preferentially interacted with a novel rather than a familiar object, GSK3 KI mice failed to discriminate between novel and familiar objects. This impairment in GSK3 KI mice also was evident in the temporal order memory task, where different objects are presented at different order time. In the coordinate spatial processing task, in which the distance between objects is altered, GSK3 KI mice also displayed a deficit. However, GSK3 KI mice functioned normally in the categorical spatial processing task where the location of two objects was switched. These findings demonstrate that in some tasks hyperactive GSK3 alone is sufficient to impair cognitive performance.

Disclosures: M. Pardo: None. **R.S. Jope:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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GENCODIS EU FP7 241995

IMI EU-AIMS FP7 115300

Title: Assessing the role of euchromatin histone methyltransferase 1 (EHMT1) in dentate gyrus function using the touchscreen operant chamber

Authors: *C. A. OOMEN, M. BENEVENTO, T. JACOBS-VAN GOETHEM, H. VAN BOKHOVEN, N. NADIF KASRI, J. C. GLENNON;
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Abstract: Kleefstra syndrome (KS) is an intellectual disability syndrome, in which patients show severely disrupted daily life functioning. It is a genetic disorder, caused by heterozygous loss-of-function mutations Euchromatin Histone Methyltransferase 1 (EHMT1), a chromatin modifying enzyme. A recently created *Ehmt1*$^{+/-}$ mouse model shows delayed postnatal development and reduced behavioral activity. Of interest for intellectual disability, deficits in fear extinction learning and spatial object recognition in *Ehmt1*$^{+/-}$ mice were also observed, suggesting changes in hippocampus function. In order to better understand the nature of such cognitive deficits and to link this to putative neurobiological underpinnings, we here present performance of *Ehmt1*$^{+/-}$ mice on location discrimination and reversal (LD) test in the touchscreen operant chamber for mice. This apparatus provides a low-stress testing environment with little demand on motor skills of the animal. Specifically, LD assesses spatial reference memory and cognitive flexibility and furthermore can uncover changes in dentate gyrus dependent pattern separation. We have trained *Ehmt1*$^{+/-}$ (n=10) and wildtype (*Ehmt1*$^{+/+}$) littermates (n=10) mice to respond to a particular response location for a food reward by choosing from two white squares on the screen at 'medium' distance. After acquisition of this general learning rule, animals were tested using varying conditions of similarity, thereby addressing 'pattern separation capacity' of *Ehmt1*$^{+/-}$ mutant mice. As pattern separation is thought to depend on the structural integrity of the dentate gyrus of the hippocampus, we will further show data on changes in adult neurogenesis.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Korean Health Technology R&D Project (HI11C1183)

Wellcome Trust/Medical Research Council (089703/Z/09/Z)

Alzheimer's Research UK (ART/PG2006/5)

Title: New automated touchscreen location and object-location tasks for the mouse: Effects of lesions of the hippocampus

Authors: *C. KIM[1,2], B. A. KENT[1,2], C. J. HEATH[1,2], T. J. BUSSEY[1,2], L. M. SAKSIDA[1,2]; [1]Dept. of Psychology, Univ. of Cambridge, Cambridge, United Kingdom; [2]MRC and Wellcome Trust Behavioural and Clin. Neurosci. Institute, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Tasks such as Trial-unique Delayed Nonmatching-to-Location (TUNL) and Paired-Associates Learning (dPAL) have been developed to test spatial and object learning in automated touchscreen operant chambers. To date, these tasks have been primarily used in rats. In this study, we adapted and optimised these tasks for use in mice and confirmed the hippocampal dependency of these paradigms with bilateral excitotoxic lesions. In addition, a new version of PAL (cdPAL) is presented, in which discrimination problems are presented in two separated areas in the touchscreen chamber. For TUNL, 32 male C57Bl/6 mice were trained on the task. Based on the final performance level, the mice were assigned to a lesion or a sham group. The lesion group received bilateral dorsal hippocampal injections of NMDA before being tested for post-surgical performance. For dPAL and cdPAL, the subjects were 24 male C57Bl/6 mice; half of the mice received NMDA lesions of the hippocampus and half were shams. The number of screen locations used for TUNL was reduced from 14 locations for rats to five locations for mice. dPAL was used without major modification, but for cdPAL a divider was inserted to divide the testing chambers into two spatial contexts. When (re) acquiring the tasks after surgery, we found that in all three tasks both hippocampal lesioned and sham animals were able to learn across sessions, but only for the TUNL task was there a significant difference between lesion and shams groups. When the performance of the last three sessions of (re) acquisition was averaged, we found that hippocampal lesions significantly impaired performance in TUNL and also dPAL, but not in cdPAL. However the number of errors committed per session was significantly higher in the lesion group in all three tasks, confirming a role for the dorsal hippocampus in these tasks. In summary, we have successfully developed and optimised touchscreen tasks to examine spatial and object-location learning and memory in the mouse. As in the rat version of the tasks, intact hippocampal function is required for normal task performance.

Disclosures: **C. Kim:** None. **B.A. Kent:** None. **C.J. Heath:** None. **T.J. Bussey:** F. Consulting Fees (e.g., advisory boards); Campden Instruments, Ltd. **L.M. Saksida:** F. Consulting Fees (e.g., advisory boards); Campden Instruments, Ltd..

Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Grant 089703/Z/09/Z

Title: Implementing repetitive, vigorous and sustained lever-like responding in the rodent touchscreen apparatus

Authors: *C. J. HEATH, L. M. SAKSIDA, T. J. BUSSEY;
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Abstract: The touchscreen testing apparatus has substantially enhanced the range of cognitive assessments that can be performed in laboratory rodents. These paradigms are designed to have high face validity with the computerised cognitive assessments conducted in humans, therefore enhancing the translational potential of findings derived from rodent behavioural studies. The touchscreen methodology also provides an automated, high throughput and standardised approach to examining rodent cognition which relies exclusively on appetitive reinforcement. A variety of tests to assess a wide range of cognitive functions have now been developed for use in this apparatus, examining domains such as working memory, pattern separation, visual discrimination, reversal and attention. However, all of these tasks require a single touch to the screen to indicate a response to a stimulus, usually in the context of a simultaneous choice procedure, and therefore it is unclear whether it is possible to elicit repetitive, vigorous and sustained lever-press or nose poke-like behaviour in this apparatus. It may not be: with the standard infra-red (IR) touchscreen, rodents do not actually have to touch the screen; approaching the screen with the snout close enough to trigger the IR beams is enough to register a response. In this sense the touchscreen is unlike traditional manipulanda such as levers. However, if such behaviour could be elicited in rodents using touchscreens, it would mean that a whole range of tasks involving fixed, variable and progressive reinforcement schedules could then be implemented in the touchscreen system, expanding the utility of the methodology, allowing such tasks to be carried out in the same apparatus as learning, memory, attention and

other cognitive tests, thus facilitating comparison between tasks. Here we report on the implementation of fixed- and progressive-ratio schedules in adult male C57BL/6 mice in the touchscreen apparatus. Fixed Ratio (FR) 1, FR3 and FR5 schedules were readily performed by the mice, as was progressive ratio (PR). Importantly, using a PR4 schedule, mice reached breakpoints of approximately 50 responses for a single reward, showing that repetitive, vigorous and sustained lever press-like behaviour can indeed be implemented in the touchscreen. Indeed, the PR task, along with appropriate control conditions, could serve as a new test - of motivation - that can be implemented using this system. The ability to implement tests of motivation and other tests requiring repetitive sustained responding using the same apparatus as that used for cognitive testing provides a substantial enhancement to the utility of the touchscreen system.

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Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Topic: F.02. Animal Cognition and Behavior

Support: EU FP7 Programme SYNSYS 242167

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SHEFC (Scottish Higher Education Funding Council)

Title: Paradoxically enhanced cognitive functions and dissociation with synaptic physiology in heterozgyous Dlg4/PSD95 mutant mice

Authors: A. E. HORNER[1], N. O. AFINOWI[1], T. J. BUSSEY[2], L. M. SAKSIDA[2], *S. G. GRANT, FRSE[3], M. V. KOPANITSA[1];
[1]Synome Ltd., Cambridge, United Kingdom; [2]Dept. of Psychology, Univ. of Cambridge, Cambridge, United Kingdom; [3]Dept. of Clin. Neurosci., Edinburgh Univ., EDINBURGH, United Kingdom

Abstract: Postsynaptic scaffold proteins in the Dlg/MAGUK (Discs large/membrane-associated guanylate kinase) family are essential for forms of learning and synaptic plasticity[1,2,3]. A recent

study[3] using a touchscreen battery to assess cognitive functions showed homozygous *Dlg4/*PSD95 mutant mice were unable to complete a basic instrumental learning task, which is a prerequisite for testing on the rest of the touchscreen battery. This drastic impairment contrasted with no alteration in this type of learning in mice lacking *Dlg2/*PSD93 and *Dlg3/*SAP102. To test whether the severe impairment in *Dlg4/*PSD95 mice was dose-dependent, we examined heterozygous mice in the present study. In contrast to the impairments in homozygous mice, heterozygous mice demonstrated normal basic instrumental learning. This allowed further testing of cognitive functions in these mice, which revealed *enhancement* in several aspects of cognition. Because previous electrophysiological studies in hippocampus slices showed homozygous *Dlg4/*PSD95 mutant mice had enhanced paired-pulse facilitation, enhanced long-term potentiation and reduced field excitatory postsynaptic potentials in the CA1 area of hippocampal slices[1,2], we asked if synaptic physiology was altered in heterozygous animals. We found that heterozygous mice showed similar synaptic phenotypes to the homozygous mice, albeit of a smaller magnitude. Thus, synaptic physiology did not correlate with changes in instrumental learning. Our study demonstrates that touchscreen tests and slice physiology are useful for examining dosage-sensitive gene effects relevant to disease models. Recent studies show that in schizophrenia, the proteins assembled with *DLG4*/PSD95 are enriched in mutations[4,5], which may now be explored using these techniques. 1. Migaud et al, Nature 1998. 2. Carlisle et al, J. Physiol. 2008. 3. Nithianantharajah et al, Nat. Neurosci. 2013. 4. Fromer et al, Nature 2014. 5. Purcell et al, Nature 2014. Supported by EU FP7 Programmes No. 242167 (SYNSYS) and No. 241995 (GENCODYS), and the SHEFC (Scottish Higher Education Funding Council).

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Poster

750. Animal Models: Recognition Memory and Novelty Detection

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Topic: F.02. Animal Cognition and Behavior

Support: European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initiative under grant agreement n115008

Title: Facilitation of spatial working memory performance following prefrontal infusion of adrenergic alpha1 agonist

Authors: *M. HVOSLEF-EIDE[1], B. M. FISHER[2], C. A. OOMEN[3], T. W. ROBBINS[2], L. M. SAKSIDA[2], T. J. BUSSEY[2];
[2]Psychology, [1]Univ. of Cambridge, Cambridge, United Kingdom; [3]Cognitive Neurosci., Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: Prefrontal adrenergic receptors have been extensively linked to spatial working memory performance. Specifically, agonists of the alpha1 and alpha2 adrenergic receptors have been shown in aged (naturally catecholamine depleted) rats to cause impairments and enhancements of spatial working memory performance respectively. It is unclear, however, whether these findings extend to young rats with a different baseline level of adrenergic firing. The current study sought to establish whether spatial working memory could be modulated in young rats through alpha1 and alpha2 agonists microinfused into the medial prefrontal cortex. The touchscreen continuous Trial-Unique Non-matching to Location (cTUNL) task was used to optimise the translational value of the study. Infusions of the alpha1 agonist phenylephrine significantly improved performance at longer delays, whilst no effect on performance was observed following infusions of the alpha2 agonist guanfacine. The study suggests that the alpha1 receptor, rather than the alpha2 receptor, plays a role in agonist-induced cognitive enhancements when spatial working memory is challenged in young, healthy rats.

Disclosures: **M. Hvoslef-Eide:** A. Employment/Salary (full or part-time):; University of Cambridge. **B.M. Fisher:** None. **C.A. Oomen:** A. Employment/Salary (full or part-time):; Radboud University Medical Centre. **T.W. Robbins:** A. Employment/Salary (full or part-time):; University of Cambridge. 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.; Eli Lilly, Lundbeck, European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initative under Grant Agreement n115008. F. Consulting Fees (e.g., advisory boards); Cambridge Cognition, Eli Lilly, Lundbeck, Teva, Shire Pharmaceuticals, Merck Sharp, Dohme, GSK Editorial: Springer Verlag. **L.M. Saksida:** A. Employment/Salary (full or part-time):; University of Cambridge. 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.; European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initative under Grant Agreement n115008. F. Consulting Fees (e.g.,

advisory boards); Campden Instruments. **T.J. Bussey:** A. Employment/Salary (full or part-time):; University of Cambridge. 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.; European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initative under Grant Agreement n115008. F. Consulting Fees (e.g., advisory boards); Campden Instruments.

Poster

751. Oscillations and Learning

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 751.01/TT92

Topic: F.02. Animal Cognition and Behavior

Title: Serotonergic modulation of septo-hippocampal theta activity during spatial learning

Authors: B. GUTIÉRREZ-GUZMÁN[1,2], J. HERNÁNDEZ-PÉREZ[1], M. LÓPEZ-VÁZQUEZ[1], *M. OLVERA-CORTES[1];
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Abstract: Theta activity (4-12 Hz) has been related to the processing of spatial information and the formation of hippocampus-dependent memory. The medial septum (MS) plays an important role in the control and coordination of theta activity as well as in the modulation of learning. It has been established that increased serotonergic activity may desynchronize theta activity, while the decrease in serotonergic activity produces continuous and persistent theta activity in the hippocampus. We want to know if the serotonin acting on medial septum to modify the learning and the functional relationship between septal and hippocampal theta activity underlying. Theta activity was recorded in the dorsal hippocampus (CA1 and DG regions) and MS of Sprague-Dawley male rats, during the execution of a test of spatial learning in the Morris water maze. In the experimental group the septal serotonin was depleted by application of 5,7 DHT (5,7-dihydroxytryptamine). The medial septal serotonin reduction facilitated learning and increased the frequency (from 6.5 to 8.5 Hz) of the hippocampal theta activity during the first days of training. Also, the coherence between MS-GD and MS-CA1 was higher in the septal serotonin depleted group mainly during de first day of test compared to control group. However,

differences in coherence were no longer observed on day 6, when is already established learning. We showed that the reduction of septal serotonin facilitates the acquisition of spatial information in association to higher functional coupling of medial septum with hippocampus. Serotonin in the medial septum could be modulating the hippocampal function.

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Poster

751. Oscillations and Learning

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grant R01MH093807

NIH grant R01MH080007

Title: Entorhinal inter-laminar coupling underlies the encoding and recognition of visual stimuli

Authors: *N. J. KILLIAN[1,2], E. A. BUFFALO[3,4];
[1]Neurosurg., Massachusetts Gen. Hosp., Boston, MA; [2]Neurosurg., Harvard Med. Sch., Boston, MA; [3]Physiol. and Biophysics department, [4]Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA

Abstract: Rhythmic activity in the hippocampal formation has been shown to support memory processes through a variety of mechanisms (Jutras et al., 2009; Girardeau et al., 2009; Rutishauser et al., 2010; Jutras et al., 2013). It was recently demonstrated that slow and fast sub-bands of the gamma rhythm route separate streams of information through the hippocampal formation (Colgin et al., 2009) and differentially coordinate place cells (Bieri et al., 2014). Specifically, in rats, it was suggested that these distinct gamma rhythms could couple different hippocampal regions to subserve encoding versus recall. Because the superficial layers of the EC provide the majority of the input to the hippocampus while the deep layers receive hippocampal feedback, inter-laminar coupling in different gamma frequencies may be associated with distinct memory processes. To test this hypothesis, we recorded simultaneously from all layers of the EC in two monkeys performing a free-viewing recognition memory task in which each image has both an encoding (novel) phase and a recognition (repeated) phase (Jutras and Buffalo, 2010;

Killian et al., 2012). Local field potential (LFP) recordings (N=210) were localized to individual laminae through registration of current source density (CSD) profiles with histology. Granger causality (GC) analysis of CSD interactions between laminae (Bollimunta et al., 2008) revealed that superficial (layers I-III) and deep layers (layers V-VI) are coordinated via rhythms in different gamma sub-bands to support visual memory encoding versus recognition: novel images were encoded through inter-laminar communication in a fast gamma band (90-130 Hz) and image recognition occurred through communication in a slow gamma band (30-80 Hz). The statistical significance of these sub-bands was verified using a cluster-based permutation test comparing the encoding and recognition conditions ($P<0.05$). With respect to baseline, on average, a 28% increase in GC in the slow gamma band occurred during recognition, compared to a 5% decrease during encoding. In the fast gamma band, a 12% increase in GC was seen during encoding, compared to a 5% increase during recognition. These results are consistent with the hypothesis suggested by Colgin et al. and provide a direct demonstration that the hippocampal formation dynamically switches population communication frequency for distinct memory processes. This scheme would reduce functional crosstalk, i.e. the unwanted mixing of encoding and recognition signals. It follows that differential communication in gamma sub-bands may be utilized by the hippocampal formation to improve the efficiency of recognition memory processes.

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Poster

751. Oscillations and Learning

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Topic: F.02. Animal Cognition and Behavior

Support: Sloan Foundation

Title: Grid cells in superficial layers of medial entorhinal cortex replay waking activity patterns during subsequent rapid eye movement sleep

Authors: ***S. G. TRETTEL**[1], L. L. COLGIN[2];
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Abstract: Hippocampal place cell sequences that activate during waking are reactivated, or 'replayed', during later rapid eye movement sleep (REM) (Louie and Wilson, Neuron 2001).

During theta rhythms in REM, information flows from superficial layers of entorhinal cortex (EC) to hippocampus, raising the question of whether EC transmits meaningful information to hippocampus during REM. Superficial layers of medial entorhinal cortex (MEC) contain grid cells, which transmit spatial information to hippocampal place cells during active waking behaviors. However, it is unknown whether grid cells reactivate this spatial information during sleep. Here, we tested the hypothesis that MEC superficial layer grid cells replay spatial sequences from waking during REM. We recorded single units and local field potentials from MEC superficial layers in rats. To identify grid cells, we recorded as rats explored a 1 m by 1 m box. Rats then ran on a 2 meter linear track to activate grid cell firing sequences. We then recorded during the 12 hour period encompassing the rats' sleep cycle. REM epochs were detected as periods when the theta (5-10 Hz) to delta (2-4 Hz) power ratio was significantly elevated. Video recordings were used to verify that rats were sleeping and exhibiting the slight limb movements that characterize REM. To search for replay, we used a method similar to the one described for place cell replay during REM (Louie and Wilson, Neuron 2001). Sequences of spikes from simultaneously active grid cells recorded during laps on the linear track were used to form templates. Templates were then compared to the grid cells' activity patterns during later REM epochs >60 s. Replay events were identified as REM periods in which grid cell ensemble activity was significantly correlated with a template. To be considered significant, correlations had to exceed 95% of correlations from 200 shuffled data sets for 4 different shuffling procedures (i.e., 800 shuffled data sets). In 59 REM episodes from 3 rats, 77% of REM episodes contained significant replay events, with 66% of laps replayed. When cell order in the templates was shuffled, significant replay events were detected in only 43% of REM episodes, with only 38% of laps replayed. These findings raise the possibility that replay occurring upstream from hippocampus in MEC can select which spatial memories are replayed by hippocampal place cells. We thank Ila Fiete for helpful discussions that motivated this work.

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Poster

751. Oscillations and Learning

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Topic: G.04. Physiological Methods

Support: Wellcome Trust grant

European Research Council grant

Title: Closed-loop optogenetic control of hippocampal gamma oscillations *in vitro*

Authors: *D. A. KUZMIN[1], T. E. AKAM[2], E. NICHOLSON[1], I. OREN[1], F. CARPENTER[1], D. M. KULLMANN[1];
[1]Inst. of Neurol., Univ. Col. London, London, United Kingdom; [2]Champalimaud Ctr., Lisbon, Portugal

Abstract: Network oscillations in the brain are a strong candidate mechanism to modulate the flow of information among anatomically connected regions. Available evidence for this hypothesis is however largely correlational. A direct test however would require a tool to manipulate the frequency, amplitude and phase of population oscillations without preventing neurons from encoding information in a population rate code. Optogenetics enables temporally precise manipulation of populations of neurons. In order to test its potential use to manipulate on-going oscillations we expressed the red-shifted excitatory opsin C1V1 in rat hippocampal pyramidal neurons, and prepared hippocampal slices for *in vitro* recordings. A gamma oscillation was elicited by applying slowly increasing illumination via a light-emitting diode (LED), and monitored via an extracellular electrode positioned in the pyramidal cell layer in CA3 or CA1. The LED driver current was then modulated according to the instantaneous local field potential (LFP). This method allowed the population oscillation to be either suppressed or amplified, depending on the LFP-LED driver current transform. Informed by a neural mass model, we further achieved dissociable control of oscillation amplitude and frequency. This 'oscillation clamp' may prove versatile in probing the behavioural role of population oscillations, especially in relation to their proposed roles in modulating functional connectivity in the brain.

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Poster

751. Oscillations and Learning

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Topic: F.02. Animal Cognition and Behavior

Support: 5R01-HD059852

Title: Phase-reversal in the oscillatory entrainment of neural interactions is a general principal of learning

Authors: *B. L. GRANNAN[1], M. ESCALONA[2], R. HASLINGER[2], Z. WILLIAMS[2];
[1]Neurosurg., Massachusetts Gen. Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: The ability to create new memories is thought to be based on the formation of synaptic-based interaction between neurons. While changes local field rhythms, thought to represent neural dendritic activity, have also been broadly observed during learning, how these rhythms relate to the interaction between neurons is unknown. Here, we used simultaneous neuronal recordings and network-modeling techniques to identify the complete correlated spiking structure of distinct cortical populations as they related to the surrounding local field rhythms in monkeys performing two unique learning tasks. We find that, pairwise interactions between neurons, as inferred from their spike couplings, were largely confined to the peak phase of the theta and alpha rhythms. Once learning occurred, however, these interactions disappeared and shifted to the trough of the cycle. These changes in the correlated spike structure of the network were not explained by changes in the spike-field coherence or periodicity of individual neurons. Moreover, they were consistently observed during both instrumental and declarative learning across four monkeys, and across both motor and prefrontal cortical sites. These findings suggest that the transition of spike interactions from the peak phase of the cortical field rhythm to its trough is a general principal of learning.

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Poster

751. Oscillations and Learning

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grant DC007702

NIH training grant R90DA033463

Title: Stochastic cortical population dynamics precisely predict taste ingestion-rejection decisions

Authors: *N. MUKHERJEE[1], J. X. LI[2], D. B. KATZ[2];
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Abstract: Taste processing in the rat gustatory cortex has been described to evolve through a series of distinct single neuron firing rate 'epochs'. The last such epoch, marked by firing rate modulations which correlate with stimulus palatability, is seen to gradually emerge in the period leading up to ~1.2 s after taste delivery to the tongue. Our recent data suggest, however, that this information might actually appear in a much more sudden and coherent ensemble firing rate transition preceding the initiation of ingestion-rejection mouth movements. In this study, we test the hypothesis that the timing of palatability-related orofacial behaviors can be accurately predicted by the timing of sudden transitions in neural ensemble firing rates. Using Hidden Markov Model (HMM) analysis of 5 gustatory cortex neural ensembles (5-25 simultaneously-recorded neurons each, 64 neurons in all from 4 rats), we find that ensemble state transitions times correlate significantly with the initiation of orofacial behaviors recorded through electromyography (EMG) from the rat jaw. These results add credence to a model of top-down modulation of brainstem generated rhythmic ingestion-rejection behaviors by the gustatory cortex, and support the idea that trial-to-trial variability seen in neural ensemble responses, often dismissed as noise, might possibly code for the initiation of palatability-related behavior on a trial-by-trial basis.

Disclosures: N. Mukherjee: None. **J.X. Li:** None. **D.B. Katz:** None.

Poster

751. Oscillations and Learning

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Training Award in Systems and Integrative Neuroscience, Grant T32 MH019524.

Title: Segregation of oscillatory processing roles for interneurons in ca1 in awake behaving animals

Authors: *S. KEELEY, J. RINZEL, A. A. FENTON;
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Abstract: Gamma oscillations in the local field potential (LFP) of the hippocampus CA1 pyramidal cell layer coordinate and segregate the spike timing of principal cells into functionally-distinct subgroups, indicating that slow 30-50 Hz gamma oscillations and fast 60-90 Hz oscillations may define distinct information processing regimes. Neural network models of CA1 motivated our hypothesis that two classes of interneuron with distinct time constants of postsynaptic inhibition are sufficient to define the two gamma regimes. The hypothesis is supported by the fact that morphological and physiological evidence indicate that the time constant of post-synaptic inhibition on pyramidal cells differ between interneuron subtype and interneurons of different subtype are preferentially innervated by distinct inputs. To help evaluate this hypothesis we analyzed extracellular tetrode recordings taken from Gyorgy Buzsaki and Bob Muller's labs of freely-moving rats and studied the spiking behavior of over 300 interneurons in relation to a nearby LFP signal. We add to previous work that has indicated that interneurons can be grouped into distinct classes based on theta phase preference (Czurko et al 2011). We show how the majority of interneurons exhibit a phase preference in a particular half-cycle of the theta phase. Despite this, the depth and shape of the theta modulation vary between interneurons, indicating phase preference as well as other modulation properties may help segregate interneuron subtype. We furthermore find that some interneurons primarily exhibit phase-locking preferences in either the slow or fast gamma regime, suggesting that distinct interneurons may be differentially involved in one or both of these regimes. We relate an interneuron’s gamma phase-locking properties in fast and slow gamma regimes with theta preferences to classify subsets of cells that may serve distinct processing purposes. These data are consistent with the hypothesis that distinct interneurons may support the local generation of slow and fast gamma oscillations in the CA1 pyramidal layer in awake behaving animals and form functionally distinct subgroups coordinated by the theta rhythm.

Disclosures: **S. Keeley:** None. **J. Rinzel:** None. **A.A. Fenton:** None.

Poster

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Program#/Poster#: 751.08/UU7

Topic: F.02. Animal Cognition and Behavior

Support: NSF 0090451

Title: Novel acoustic stimuli can alter locomotor speed-theta relationship across the septotemporal axis of the hippocampus

Authors: *L. L. LONG[1], A. A. NORRIS[2], J. R. HINMAN[3], C.-M. CHEN[1], I. H. STEVENSON[1], H. L. READ[1,2], M. A. ESCABI[2], J. J. CHROBAK[1];
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Abstract: Evidence links single unit hippocampal responses to the detection of novel sensory variables (e.g. auditory); however, few studies have systematically explored the response of large-scale network activity, such as hippocampal theta oscillation (6-12 Hz) to auditory stimuli. The hippocampal theta rhythm has been linked to the execution of ongoing motor programs, cognitive processes such as encoding and retrieval, as well as environmental manipulation (novelty). The theta signal exhibits significant variation across the laminar, proximodistal (transverse) and septotemporal (longitudinal) axes. Previously, we demonstrated that while navigating in novel, physical space, the relationship between speed and theta power increased at all CA1 sites along the septotemporal axis. This novelty related increase in theta power habituated within/across multiple recording sessions in a single day, where habituation at temporal CA1 sites was more prominent as compared to septal sites. Given these findings, we ask -- beyond physical space, whether acute and passive sound delivery [broadband (0-40kHz) white noise] alters septotemporal theta indices and whether theta dynamics habituate with repeated sound exposures (experience). Ten Long-Evans rats were trained to run a rectangular maze for food reward and were each outfitted with 16 electrodes spanning the septotemporal axis of septal CA1. Preliminary analyses indicate a location specific decrease in the slope of the speed-theta relationship in septal hippocampus in response to passive delivery of novel acoustic signals while the rats traversed a very familiar environment. Further, this location specific decrease habituates (return to baseline) across repeated sound exposures across days. Ongoing analyses will verify how changes in theta activity relate to 1) differences in behavior (speed, acceleration while navigating the maze), and 2) functional differentiation across the septotemporal CA1 axis of the hippocampus. While preliminary, the current findings suggest that the hippocampal theta signal exhibits amplitude and frequency variation in response to acoustic manipulations and allow for subsequent evaluation of how that change varies in relation to repeated modification of the acoustic environment.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: RIKEN Brain Science Institute

The Picower Institute Innovation Fund

Human Frontier Science Program (HFSP)

Title: Successful execution of working memory linked to synchronized high frequency gamma oscillations

Authors: *J. YAMAMOTO, J. SUH, D. TAKEUCHI, S. TONEGAWA;
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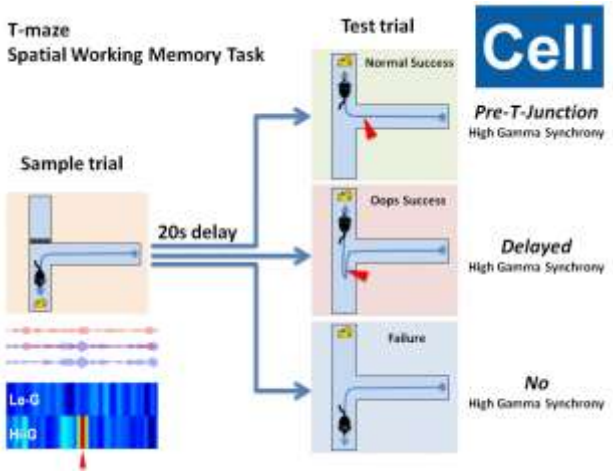
Abstract: Network oscillations are proposed to underlie the temporal binding of spatially distributed neuronal populations to enable information processing for cognition and its ensuing behavior. In addition, certain oscillations are hypothesized to allow conscious perception and awareness of associations between external cues and internal goals encoded in the synchronized brain areas. In particular, gamma oscillations correlate with perception, memory and attention. It has been hypothesized to play an important role in cognition and its ensuing behavior, but evidence that links a specific neuronal oscillation to a discrete cognitive event is largely lacking. We measured neuronal activity in the entorhinal-hippocampal circuit while mice performed a reward-based spatial working memory task. During the memory retention period, a transient burst of high gamma synchronization preceded an animal's correct choice in both prospective planning and retrospective mistake correction, but not an animal's incorrect choice. Optogenetic inhibition of the circuit targeted to the choice point area resulted in a coordinated reduction in both high gamma synchrony and correct execution of a working memory-guided behavior. These findings suggest that transient high gamma synchrony contributes to the successful execution of spatial working memory. Furthermore, our data are consistent with an association between transient high gamma synchrony and explicit awareness of the working memory content.



Yamamoto et al., Successful Execution of Working Memory Linked to Synchronized High-Frequency Gamma Oscillations, Cell (2014), http://dx.doi.org/10.1016/j.cell.2014.04.009

Disclosures: J. Yamamoto: None. **J. Suh:** None. **D. Takeuchi:** None. **S. Tonegawa:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH F30 (NRSA)

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Title: Spatial sequences during theta rhythms in the hippocampus are differentially modulated by slow and fast gamma rhythms

Authors: *K. W. BIERI, L. L. COLGIN;
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Abstract: The principal neurons of the hippocampus, called ‘place cells’ (O’Keefe, Exp Neurol 1976), fire when an animal is in a specific location of an environment, and are thought to represent the ‘where’ component of episodic memories. When an animal traverses a set of

locations, place cells with spatial receptive fields ('place fields') in these locations fire in sequences. Such spatial sequences are activated in a temporally compressed form during individual cycles of the ~8 Hz theta rhythm (Skaggs et al., Hippocampus 1996; Dragoi and Buzsaki, Neuron 2006; Foster and Wilson, Hippocampus 2007). These sequences vary with regard to their spatial coding properties across different theta cycles, but the reasons for this are not well understood. Recent results showed that sequence duration correlates positively with the number of cycles of corresponding gamma rhythms (~25-100 Hz) (Gupta et al., Nat Neurosci, 2012). Hippocampal gamma rhythms subdivide into 'slow' (~25-55 Hz) and fast (~60-100 Hz) variants (Colgin et al., Nature, 2009) that reflect distinct place cell coding modes (Bieri et al., Neuron 2014). To examine whether slow and fast gamma variants differentially affect spatial coding properties of place cell sequences, we recorded CA1 cell ensembles and local field potentials in freely behaving rats traversing a linear track. We found that that the large majority of significant sequences occurred in forward order. Forward sequences coded longer distances during periods of slow gamma than during periods of fast gamma. Moreover, slow gamma sequences were more temporally compressed than fast gamma sequences. In other words, place cell ensemble activity during periods of slow gamma represented longer distances in a shorter amount of time. Additionally, spikes were significantly more phase-locked to theta, and tended to occur on different theta phases, during periods of slow gamma compared to periods of fast gamma. These findings support the conclusion that slow and fast gamma reflect different spatial coding modes in the hippocampus, with slow gamma promoting temporally compressed representations of space and fast gamma supporting representations that more closely match animals' spatial experiences in real time.

Disclosures: K.W. Bieri: None. **L.L. Colgin:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS-1121969

Title: Differences in hippocampal single-unit response profiles during theta-contingent eyeblink classical conditioning early and late in training

Authors: *J. J. CICCHESE[1], R. D. DARLING[2], S. D. BERRY[1];
[1]Psychology and Ctr. for Neurosci., Miami Univ., Oxford, OH; [2]Univ. of Mississippi Med. Ctr., Jacksonville, MS

Abstract: The hippocampus is an essential structure in the acquisition of trace eyeblink classical conditioning (EBCC). Hippocampal neurobiological oscillations at theta frequency serve as an index of hippocampal functioning and are known to play an important role in this task and other forms of associative learning. Using a brain-computer interface (BCI), our lab has shown that triggering EBCC trials in the explicit presence of hippocampal theta (3-7 Hz) increases learning rate while training in its absence leads to a decrease in learning rate compared to yoked controls. Additionally, there is an established phase relationship between theta oscillations and the firing of identified single units within the hippocampus. The present study utilized the BCI with single-unit recordings to analyze the responses of CA1 pyramidal cells throughout theta-contingent trace EBCC. Units were sorted off-line and classified as putative pyramidal cells based on characteristics such as waveform duration and spike rate. Analysis of the response profiles compared firing between the theta and non-theta conditions during the early (initial acquisition) and late (asymptotic performance) phases of learning. Furthermore, multiple measures of neuron responding (i.e. magnitude of firing rate and percent of cells changing firing rate) were compared to behavioral measures to determine which is more predictive of conditioned response performance. These results serve to clarify the specific mechanism of theta involvement in optimal processing through either recruitment of cells or modulating their firing rates. Such findings have important implications for understanding the role of theta oscillations during the acquisition and asymptotic performance of conditioned behavior.

Disclosures: J.J. Cicchese: None. **S.D. Berry:** None. **R.D. Darling:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NHMRC (Australia)

Florey Foundation

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Victorian Government Operational Infrastructure Support Program

Title: A nucleus incertus-septohippocampal circuit driving theta oscillations alters behavioral state - Implications for arousal and mood regulation

Authors: *S. MA, E. E. K. E. ONG-PALSSON, D. HAWKES, R. A. D. BATHGATE, A. L. GUNDLACH;
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Abstract: The nucleus incertus (NI) of the pontine periventricular grey consists of GABAergic neurons with long-range ascending projections to forebrain. Efferent and afferent connections implicate the NI in processes of behavioral planning, habenular function, hippocampal/cortical activity in attention/memory, and oculomotor control (Goto et al., 2003). The NI is a site of corticotropin releasing-factor (CRF) action, and forms a neural circuit positioned to modulate arousal/stress responses and de/synchronization of hippocampal theta rhythm, an oscillatory activity (4-12 Hz) prominent in the electroencephalograph (EEG) during exploration and memory processes (Ma et al., 2013). Theta rhythm underlies goal-oriented behavior and cognition, and is a neurophysiological signature of REM sleep. The NI is the primary site of neurons producing the neuropeptide, relaxin-3, which contributes to theta generation and associated spatial working memory via actions on the septohippocampal system. However, NI function in awake, behaving animals remains unclear. Therefore, we used a pharmacogenetic approach (ie. designer receptors exclusively activated by designer drugs, DREADDs) to modulate NI neuron activity in adult male Sprague-Dawley rats and assessed the behavioral and physiological consequences. An adeno-associated viral vector was used to transduce a modified human muscarinic receptor, hM3Dq, into NI neurons of adult male rats. For behavioral and telemetric EEG recordings, hM3Dq was activated by the specific DREADD ligand, clozapine-N-oxide (CNO; 3 mg/kg, ip). In hM3Dq-expressing rats, NI activation by CNO produced long-lasting theta with decreased alpha and beta band activity, associated with increased locomotor activity reflected as greater and persistent horizontal activity compared to control vector rats ($n=7$-8/group; $P<0.01$), but not increased movement velocity, suggesting effects related to increased arousal and impairment of habituation and rest. Current studies are examining effects of NI activation on performance in tests of spatial and emotional memory, anxiety/aversive behavior, and associated neural activity, along with comparative studies of NI inactivation with hM4Di. Current data demonstrates that the NI is an integrative neural network regulating behavioral state associated with persistent theta oscillations and arousal.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Roche Postdoctoral Fellowship (RPF) program, F. Hoffmann – La Roche Ltd., Basel, Switzerland.

Title: High frequency neuronal oscillations in a cynomolgus macaque test of working memory following NMDA receptor antagonism

Authors: *A. V. GOONAWARDENA[1], J. HEISS[1], C. GLAVIS-BLOOM[1], E. BORRONI[2], D. ALBERATI[2], T. L. WALLACE[1];
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Abstract: Disruptions in sensory processing and abnormal temporal integration of neuronal oscillations, especially within the gamma frequency range (30-80 Hz), have been identified in schizophrenic patients during working memory tasks and may contribute to the poor performance within this cognitive domain. Experimentally, alterations in gamma oscillations as well as the induction of other schizophrenia-like symptoms including cognitive deficits can be induced with NMDA receptor antagonists (e.g., phencyclidine [PCP], ketamine) in rodents, non-human primates (NHPs) and humans. Given that NHPs and humans have homologous prefrontal cortical structures that mediate attention and working memory processes, our objective was to characterize neuronal oscillations and event-related potentials (ERPs) to assess sensory and cognitive processing in cynomolgus macaques performing a delayed-match-to-sample (DMTS) working memory task. Macaques (n=7) were trained to match a sample stimulus following a delay period on a touchscreen in exchange for food rewards. Subsequently, all subjects were implanted with EEG electrodes [placed on the dura mater above the frontal cortex (FC) and primary visual cortex (V1)]. Thereafter, all animals received acute doses of PCP (0.03, 0.056, 0.1 mg/kg) or vehicle (Veh) and the effects on DMTS performance and EEG oscillations were measured. As compared to vehicle treatment, PCP produced a significant dose-dependent decrease in DMTS performance accuracy. EEG analysis during DMTS performance demonstrated that post-stimulus high gamma (51-80Hz) oscillations were significantly enhanced by PCP in the FC during correct responding. Moreover, PCP significantly elevated the amplitude of low gamma (30-50Hz), while suppressing alpha (8-12Hz) oscillations in FC. Similarly, in V1,

PCP elevated both low and high gamma oscillations. Furthermore, PCP showed a significant post-stimulus enhancement in high gamma and reduction in beta (16-24Hz) bands during correct responding. In addition, PCP significantly prolonged the cognitively-relevant P300 component of the mean ERP during correct responses in FC but not in V1. Overall, our results suggest that acute administration of a NMDA receptor antagonist disrupts neuronal oscillations and cognitive processing, especially in the FC, and this may contribute to impaired cognitive performance in macaques. This study may help to define the role of high frequency oscillations in cognitive processes in higher order species, and to enhance our understanding of EEG recordings as a translatable biomarker for cognitive impairments associated with schizophrenia.

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Poster

751. Oscillations and Learning

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH100820

Title: Theta-rhythmic drive between medial septum and hippocampus in slow wave sleep and microarousal: A Granger causality analysis

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[1]J. Crayton Pruitt Family Dept. of Biomed. Engineering, Univ. of Florida, Gainesville, FL;
[2]Dept. of Psychiatry, BIDMC, Harvard Med. Sch., Boston, MA

Abstract: Medial septum (MS) plays a critical role in controlling the electrical activity of the hippocampus (HIPP). Rhythmic firing of MS neurons in synchrony with HIPP theta oscillations was reported during waking motor activity in freely moving rats and in REM sleep and in response to arousing stimuli in head-restrained rats. Less is known about MS-HIPP interactions in non-theta states, although it was noted in early reports that MS burst activity may remain unchanged when HIPP theta rhythm is temporally replaced by other type of activity and that

theta rhythmic MS cell decrease firing during HIPP sharp waves. The present study used Granger causality (GC) to identify the direction of rhythmic influence between MS and HIPP within the theta frequency band during slow wave sleep and microarousals. MS neuron firing was recorded using three tetrodes connected to separate microdrives in freely moving rats, along with HIPP field potentials and neck muscle EMG. MS neurons (>50) were identified in 4 rats using principal component and K-means clustering algorithms. Spectral analysis included autospectra of HIPP field potentials, and MS neuron firing, as well as MS-HIPP coherence and GC in both MS->HIPP and HIPP->MS directions using a non-parametric algorithm specifically developed for handling point processes (spike trains). The majority of MS units were rhythmically firing in theta states and had a dominant theta peak in their autospectra (6.2+0.12 Hz) during slow wave sleep (SWS) as well. Importantly, while MS-HIPP theta coherence is significant, and GC revealed a unidirectional MS->HIPP influence over a wide band (2-10Hz) with a maximum at 8.2+2.58 Hz, there was no theta peak in the hippocampal autospectra; HIPP->MS was insignificant. In contrast, during microarousals, theta peaks were dominant in both MS and HIPP autospectra, and elevated MS-HIPP theta coherence was accompanied by bidirectional GC where MS->HIPP and HIPP->MS theta drives were of equal magnitude. The present findings suggest a modification of our understanding of the role of MS as the ultimate theta generator in two regards. First, a MS->HIPP theta drive does not necessarily induce theta oscillations in the hippocampus, as found in SWS. Second, HIPP theta oscillations involve bidirectional rhythmic interactions between MS and HIPP.

Disclosures: **D. Kang:** None. **M. Ding:** None. **I. Topchiy:** None. **L. Shifflett:** None. **B. Kocsis:** None.

Poster

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Support: NIH Grant R01MH093807

NIH Grant R01MH080007

Title: Theta activity in the monkey hippocampus during virtual navigation

Authors: *M. J. JUTRAS[1,2], J. A. SOLYST[1,3], E. A. BUFFALO[1,2];
[1]Physiol. and Biophysics, [2]Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA; [3]Laney Grad. Sch., Emory Univ., Atlanta, GA

Abstract: The hippocampal theta rhythm, a high-amplitude oscillation usually occurring within the range of 4-8 Hz in rodents, is most often observed when animals engage in exploratory behavior. Theta has recently gained increased attention thanks to reported observations in bats and humans, two species which possess different mechanical means of spatial exploration (namely, echolocation and visual exploration, respectively). Our lab has published observations of a theta-like oscillation in the hippocampus and entorhinal cortex of rhesus macaque monkeys (Killian et al., 2012; Jutras et al., 2013). This oscillation, consisting of bouts of oscillatory activity in the 3-12 Hz range, occurs during free-viewing of visual stimuli, and undergoes a phase reset concurrent with the onset of each saccade, suggesting a close link between theta and the mechanical means of sampling sensory information in the environment. Notably, other studies of hippocampal recordings in the awake monkey have reported a lack of prominent theta oscillations. One potential explanation for this discrepancy is that the presence of theta is highly dependent on task demands, requiring active exploration of stimuli in the environment. Recently, we trained a macaque monkey to navigate a virtual environment using a joystick, performing a basic foraging task for reward. Here, we report preliminary results from hippocampal recordings during virtual navigation, showing evidence of high-amplitude theta oscillations that are strongly attuned to the rate of saccadic eye movements. The average theta peak was at 4 Hz, but the frequency of theta was highly correlated with saccade rate within the range of 3-5 Hz. Interestingly, prominent theta oscillations were present even during periods where no forward movement occurred, as long as saccadic eye movements were evident. These periods typically consisted of epochs when the monkey paused in one spot in the environment, and was turning to explore the environment in search of the next reward. These data are consistent with a recent finding of high theta power when rats make exploratory head-scanning movements during pauses in locomotion (Monaco et al., 2014). In addition, we found evidence of cross-frequency coupling between theta phase and the amplitude of gamma-band (30-120 Hz) oscillations, with higher amplitude gamma occurring during the falling phase of theta. These results provide further evidence that theta oscillations in mammalian species may act as a general mechanism to link hippocampal activity with active sensory mechanisms occurring during exploratory behavior.

Disclosures: **M.J. Jutras:** None. **J.A. Solyst:** None. **E.A. Buffalo:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: W. M. Keck Foundation grant for pioneering research

Title: New signal processing method reveals discrete structure of local field potential in hippocampus

Authors: *Y. A. DABAGHIAN[1], J. DEVITO[2];
[1]Neurol. and pediatrics, Jan and Dan Duncan Neurolog. Res. Institute, Baylor Col. of Med., Houston, TX; [2]CAAM, Rice Univ., Houston, TX

Abstract: A physiological interpretation of the biological rhythms, e.g., the local field potentials (LFP) depends on the mathematical and computational approaches used for its analysis. Most mathematical methods of the LFP studies are based on braking the signal into a combination of simpler components, e.g., the sinusoidal harmonics of Fourier analysis or the wavelets in Wavelet Analysis. However, a common feature of most signal decomposition methods is that their prime components are presumed from the onset, and the goal of the subsequent analysis reduces to identifying the combination that best reproduces the original signal. We propose a fundamentally new method, based on a number of deep theorems of complex function theory, in which the prime components of the signal are not *a priori* presumed, but discovered empirically. Applying this method reveals a fundamentally new structure in the hippocampal LFP signals in rats in mice. In particular, our current results suggest that the LFP oscillations may consist of a superposition of a small, discrete set of phase modulated oscillatory processes, which may better capture the signal's actual physical structure, i.e., the pattern of synchronous activity in neuronal ensembles. Proving this hypothesis will help enormously to advance a principal, theoretical understanding of the neuronal synchronization mechanisms. We anticipate that it will reveal new information about the structure of the LFP and other biological oscillations, which should provide insights into the underlying physiological phenomena and the organization of brains states that are currently poorly understood, e.g., sleep and epilepsy.

Disclosures: Y.A. Dabaghian: None. **J. DeVito:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Klingenstein Fund

Sloan Foundation

Title: The correlation between gamma frequency and running speed differs for slow and fast gamma rhythms in freely behaving rats

Authors: *C. ZHENG[1], K. W. BIERI[2], S. G. TRETTEL[2], L. L. COLGIN[3];
[2]Inst. for Neurosci., [3]Ctr. for Learning and Memory, [1]Univ. of Texas At Austin, Austin, TX

Abstract: Gamma rhythms (~25-100 Hz) are thought to play an important role in spatial memory processing in the hippocampus. Previous work has shown that the frequency of gamma activity in rat hippocampus increases with running speed (Ahmed and Mehta, J Neurosci 2012). This is believed to allow faster transitions between sequences of gamma-modulated place cells at higher running speeds. However, increasing evidence suggests that gamma rhythms split into distinct slow (~25-55 Hz) and fast (~60-100 Hz) gamma subtypes. Slow gamma synchronizes hippocampal subfield CA1 with inputs from neighboring subfield CA3, whereas fast gamma couples CA1 with inputs from the medial entorhinal cortex (MEC) (Colgin et al., Nature, 2009). Thus, it is possible that frequencies within the distinct slow and fast gamma ranges show different correlations with running speed. In order to address this hypothesis, we recorded local field potentials in CA1 (n=4), CA3 (n=4), and MEC superficial layers (n=3) in rats running on a linear track. We fit 1-3 normal probability density functions to gamma power distributions for frequency versus running speed and estimated parameters using the expectation maximization method applied to theta phase precession data previously (Yamaguchi et al., J Neurophysiol, 2002). The data were best fit by two distinct components, which corresponded to strong slow gamma during low running speeds and strong fast gamma during high running speeds. In CA1, the fast gamma component was more dominant than the slow gamma component. In CA3, the slow and fast gamma components were weighted approximately equally. In the superficial layers of MEC, a fast gamma component emerged at relatively high running speeds, but no slow gamma component was apparent at low running speeds. We next analyzed the correlation between gamma frequency and running speed separately for slow and fast gamma. We found a highly positive correlation between fast gamma frequency and running speed in both CA1 and MEC. Slow gamma frequency, however, did not significantly change with running speed in either CA1 or CA3. These results support the conclusion that slow and fast gamma constitute separate states in the hippocampal network, with fast gamma driven by MEC at high running speeds and slow gamma driven by CA3 at low running speeds.

Disclosures: C. Zheng: None. **K.W. Bieri:** None. **S.G. Trettel:** None. **L.L. Colgin:** None.

Poster

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Support: NIH Grant NS076763

Title: Impact of Nav1.1 knockdown on CA1 in hippocampal function

Authors: *S. SAKKAKI[1], A. C. BENDER[2], A. GULLEDGE[2], P.-P. LENCK-SANTINI[1]; [1]Neurolog. Sci., Univ. Of Vermont, Burlington, VT; [2]Neurol., Geiseil Sch. of Medecine at Dartmouth, Hanover, NH, NH

Abstract: Voltage-gated sodium channel Nav1.1 (coded by SCN1A gene) is important for maintaining fast and burst firing neuronal activity throughout the SNC. In the hippocampus, such activities are the hallmark of fast spiking GABAergique interneurons. In CA1, interneurons regulate pyramidal cells (place cells) activity and maintain hippocampal theta rhythm. The aim of this study is to investigate the consequences of Nav1.1 loss of function on the CA1, in hippocampal function. We hypothesize that Nav1.1 loss of function will lead to a drastic diminution of fast firing activity that will affect place cells properties and hippocampal oscillations. This could be the neural mechanism underlying spatial representation deficit and cognitive impairment observed in different neurological disorders (Dravet Syndrome, Autism, Alzheimer disease) associated with SCN1A mutations. Using a ShRNA interference approach, we induced a local Nav1.1 down regulation in the CA1 area in adult rats. In-vitro experiments show that NaV1.1 deficits in CA1 affect neuronal coding of interneurons. In-vivo single-unit recording freely moving rats reveals that the relationships between place cell firing and hippocampal theta rhythm has been altered. This impairment is associated with an increase of exploratory activity in a novelty recognition task related to spatial memory deficits.

Disclosures: S. Sakkaki: None. **A.C. Bender:** None. **A. Gulledge:** None. **P. Lenck-Santini:** None.

Poster

751. Oscillations and Learning

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 751.19/UU18

Topic: F.02. Animal Cognition and Behavior

Support: FIS/IMSS/PROT/G11/921

Title: The spatial learning is associated with modifications of the theta oscillations on hippocampus layers and septal complex in the rat

Authors: J. HERNÁNDEZ-PÉREZ[1,2], B. GUTIÉRREZ-GUZMÁN[1,2], M. LÓPEZ-VÁZQUEZ[1], *M. CERVANTES[3], M. OLVERA-CORTÉS[1];
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Abstract: The hippocampus generates a prominent pattern of oscillatory activity at theta frequency (4-12 Hz) which occur during the extraction of information from environment (walking, running, sniffing), while the septal complex contain pacemaker cell that fire at theta frequency. In rat has been showed that theta oscillations interruption through lesion or inactivation of the medial septum severely affects the performance of memory tasks hippocampal dependent. However, it is unknown what role play the theta oscillations on the hippocampal information processing. Recent evidence has showed that the execution of working memory as well as the improvement in the performance of a spatial memory task modifies the hippocampal theta activity. Because the septal complex is considerated as a pacemaker of hippocampal activity, we proposed that the theta activity of the septal complex can suffer changes associated to learning of a memory task. Furthermore, as each hippocampal layer can generate a theta dipole, the hippocampal circuits could generate changes on its oscillatory activity associated with the learning, independently of the septal oscillatory activity. To test our hypothesis, we evaluated through power and coherence analysis the relationship between theta oscillations of septal complex and hippocampal layers, as well as between the intrahipocampal layers during the learning of a spatial memory task. Six Sprague-Dawley rat, were implanted with two electrode arrays, one in the dorsal hippocampus that was composed for seven nichrome wires (diameter 25 microns) spaced 190 microns through the dorso-ventral axis and other in the septal complex with a three electrodes array spaced 700 microns through the dorso-ventral axis. The spatial memory task was evaluated using the Morris water maze (six trials per day for four days). The result showed that learning modified the power and coupling of theta activity between septal complex and hippocampus. During the first trials at the day one (when the learning is not established), the theta frequency peak was on 7.5 Hz, while during last trials when the learning acquisition is occurring the frequency peak was lower (6.5 Hz). However, when the learning was established,

the theta frequency peak returned to 7.5 Hz, these changes coincided with an increased coherence between septal complex and hippocampus in the same frequency. In conclusion, the learning acquisition was accompanied with a reduction at the theta frequency peak; while, after of the learning establishment the theta activity returned to its initial frequency and the coupling of theta activity in the septo-hippocampal system increased.

Disclosures: J. Hernández-Pérez: None. **B. Gutiérrez-Guzmán:** None. **M. López-Vázquez:** None. **M. Cervantes:** None. **M. Olvera-Cortés:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: AFOSR FA9550-12-1-0369

NSF award BCS-1058937

Title: Neural activity in the medial prefrontal cortex changes across a range of time scales

Authors: *Z. TIGANJ[1], J. KIM[2], M. JUNG[2], M. W. HOWARD[1];
[1]Ctr. For Memory and Brain, Boston Univ., Boston, MA; [2]Ctr. for Synaptic Brain Dysfunctions, IBS and Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: Neural firing has been argued to maintain working memory over a few seconds. Models of interval timing require a scale-invariant timing signal that maintains information about time over the range of a few seconds up to tens of minutes. We analyzed the firing rate of isolated units in rat prefrontal cortex while the animals were doing multiple trials of a temporal bisection task. To minimize the behavioral correlates we only analyzed the delay intervals when the animal was waiting in a restricted area. Within a delay period, a subset of neurons showed a peak of firing at discrete points during the delay. The distribution of peak times roughly followed a power law distribution up to a few seconds. Across delay periods, many neurons showed gradual changes in their firing rate from one trial to the next. Some neurons showed autocorrelation over a few trials, spanning a few dozens seconds, but some neurons showed autocorrelation over tens of minutes---the longest interval that could have been measured in these recordings. The distribution of autocorrelation times also resembled a power-law

distribution. These results suggest that the medial prefrontal cortex could support timing over both short time scales (a few seconds) and long time scales (tens of minutes). Moreover, the power law distributions suggest that the dynamics are scale-invariant, consistent with a range of behavioral results.

Disclosures: Z. Tiganj: None. **J. Kim:** None. **M. Jung:** None. **M.W. Howard:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC Discovery Grant

NSERC University Faculty Award

NSERC PhD fellowhip

Title: Properties and synaptic targets of calretinin-positive interneuron-specific interneurons in the mouse CA1 hippocampus

Authors: *O. CAMIRÉ, L. TYAN, S. CHAMBERLAND, E. MAGNIN, L. TOPOLNIK; IUSMQ, Quebec, QC, Canada

Abstract: Hippocampal GABAergic interneurons provide multiple sources of feedforward and feedback inhibition to principal cells in conjunction with specific excitatory inputs and in relevance to particular brain states. Moreover, a higher-level inhibitory circuitry specialized in innervating inter-neurons exists in the CA1 hippocampus. Some cells that express calretinin (CR) exhibit a remarkable and highly selective connectivity with hippocampal interneurons. These interneuron-specific (IS) cells may control the activity of different inhibitory circuits, thus allowing the coordination of the whole hippocampal network. However, the properties and functional connectivity of IS interneurons remain largely unknown. We used a combination of whole-cell recordings, optogenetics and anatomical reconstruction to identify different classes of CR-positive interneurons in the mouse hippocampus. Analysis of physiological and morphological variables revealed three types of CR-positive cells: the first type that targeted mostly the CA1 stratum radiatum, the second with an axon projecting exclusively to the O/A, and the third type of CR interneurons with wide axonal arborization that covered all CA1 layers

and projected to CA3 and subiculum. The three types of interneurons differed with respect to their firing pattern and to the presence of Ih current and rebound action potential. Among postsynaptic targets of CR-positive interneurons, we found axo-axonic cells, basket cells (both parvalbumin- and VIP-positive), bistratified cells, oriens-lacunosum/moleculare interneurons, and some long-projecting interneurons within oriens/alveus. CR-positive interneurons were negative for somatostatin, muscarinic receptor 2 and parvalbumin; however ~50% of these cells coexpressed enkephalin. Thus, combined morphological and physiological analysis revealed three subtypes of CR-positive interneurons, which may be specialized in innervating specific classes of interneurons and play different functions.

Disclosures: O. Camiré: None. **L. Tyan:** None. **S. Chamberland:** None. **E. Magnin:** None. **L. Topolnik:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 752.03/UU21

Topic: F.02. Animal Cognition and Behavior

Title: Organization of neocortical and amygdalar input into the perirhinal and entorhinal cortex

Authors: *N. L. CAPPAERT, J. G. P. WILLEMS, P. J. P. CHAMEAU, T. R. WERKMAN, W. J. WADMAN;
Univ. Amsterdam, Amsterdam, Netherlands

Abstract: The perirhinal cortex (PER) and entorhinal cortex (EC) are an intricate part of the (para)hippocampal network and they are involved in memory formation, object recognition, sensory representation and spatial orientation. The PER and EC receive input from several cortical and subcortical areas, including the insular cortex and the amygdala. We used optical imaging with voltage sensitive dyes to determine the spatiotemporal organization of neocortical and amygdalar inputs into the PER and EC in horizontal mouse brain slices. Experiments were performed in the presence of 1 μM bicuculline to slightly reduce inhibition. Electrical stimulation of the neocortical insular cortex induced synaptically evoked activity that gradually propagated through the PER to lateral EC (LEC) and finally activated the medial EC (MEC). In total, the complete rostral to caudal propagation lasted 40 - 50 ms. On the other hand, amygdala stimulation activated the deep layers of the PER and LEC first, and once the deep layers of the rhinal cortices were active, population activity propagated towards the superficial layers and to

the MEC. The amygdala activated the rhinal cortices in an intensity-dependent manner. The deep layers of the PER and EC were activated 150-200 ms after stimulation of the amygdala at low stimulus intensity (50 μA), while 500 μA stimulation induced a response with a shorter latency (25-50 ms). Low intensity stimulation (50 - 100 μA) of the amygdala failed to activate the PER and EC in an all or none fashion in 25 - 50% of the cases. This failure rate decreased with increasing stimulus intensity; with a high-intensity stimulus (500 μA) failures were no longer observed. The data shows new insights into the spatiotemporal organization of the parahippocampal region in response to neocortical and subcortical inputs, suggesting that both the neocortex and the amygdala activate the same circuitry, although in a different fashion. Next, we will perform whole-cell patch clamp recordings on neurons in the target areas. This approach will be taken to unravel the network interactions that are responsible for the specific spatiotemporal activation patterns we observed with the voltage sensitive dye imaging experiments.

Disclosures: **N.L. Cappaert:** None. **J.G.P. Willems:** None. **P.J.P. Chameau:** None. **T.R. Werkman:** None. **W.J. Wadman:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: German National Science Foundation grant DFG DE 792/4-2 to de Souza Silva.

Title: A system subsuming prefrontal cortical AMPA-R and the hippocampal CA3 area controls the integration of spatial- and temporal-order memory into episodic memory

Authors: O. Y. CHAO[1], *J. P. HUSTON[2], M. A. DE SOUZA SILVA[1];
[1]Ctr. for Behavioral Neurosci., [2]Univ. of Dusseldorf, Dusseldorf, Germany

Abstract: We applied a disconnection procedure to examine whether the circuit involving the prefrontal cortex (PFC)-hippocampus CA3 is critical for episodic-like memory and examined whether the circuit depends on glutamatergic receptors in the PFC. The neurotoxin NMDA was unilaterally injected into the medial PFC and also into the hippocampal CA3 area situated either in the ipsilateral or contralateral hemisphere. Tests for episodic memory and its components were then conducted using a task that assesses the integration of the distinct memory systems for

"what, where and when" into episodic memory, based on the integrated memory for objects in space and their temporal order (Kart-Teke et al., 2006). Disconnection of the medial PFC-CA3 circuit prevented the integration of "what-where-when" memory, but left memory for the components "what", "where" and "when", per se, intact. These results indicate that a functional circuit between PFC and the CA3 area is critical for the expression of episodic memory. Employing the same disconnection model, we injected NMDA unilaterally into the CA3 area. Either AP-5, or CNQX, was then injected into the PFC 10 min prior to the test trial. Injection of the AMPA-receptor antagonist CNQX (but not the NMDA-receptor antagonist AP-5) into the contralateral (but not ipsilateral) PFC, prevented episodic-like memory. These results indicate a functional pathway between the medial PFC and hippocampal CA3 area that determines the integration of memory for "what, where and when" components into episodic memory, but not the component memory processes, per se. Furthermore, this functional pathway depends on the AMPA, but not NMDA receptors in the PFC. These results shed light on the understanding of the neurobiological mechanism underlying the integrative processes that define episodic memory.

Disclosures: O.Y. Chao: None. **J.P. Huston:** None. **M.A. de Souza Silva:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA029809

Title: Stimulus compounding produces a "migration effect" in rats

Authors: B. J. DECORTE, *M. S. MATELL;
Psychology, Villanova Univ., Villanova, PA

Abstract: Interval timing, the perception of time in the seconds-to-minutes range, is often assessed using the peak-interval (PI) procedure. In a typical PI task, the onset of a discriminative stimulus indicates that reinforcement may be earned for responding after a set "criterion duration" has elapsed (e.g. 30 seconds). On temporally extended, non-reinforced probe trials, response rates peak around this criterion duration. Several studies using the PI procedure have shown that when rats are simultaneously presented with two cues (e.g., a tone and a light), each

associated with two different durations (e.g., 10 and 20 s, respectively), they respond as if they are timing an average of the two durations (Swanton, Gooch et al. 2009). In previous investigations of this temporal integration effect, rats have been trained to respond on a single response manipulandum (e.g., a nosepoke). We examined whether associating each cue with a single response option is necessary for temporal averaging. Rats (n=10) were trained that reinforcement was probabilistically available for the first nosepoke emitted 5 or 20 seconds after the onset of a tone or houselight, respectively. The rats were trained to respond on a left nosepoke in response to one cue/duration pair and a right nosepoke in response to the other. During final testing sessions, 20% of all trials were compound probes in which the tone and light were presented as a simultaneous compound. When the cues were presented in isolation, responses occurred on the appropriate nosepokes and were maximal near the designated criterion durations (5.24s +/- 0.95 & 23.69s +/- 3.16). In contrast, during compound probes, responses on the 5s nosepoke were shifted rightward from the tone alone trials (7.81s +/- 3.33), whereas responses on the 20s nosepoke were shifted leftward from the light alone trials (15.99s +/- 2.14). As the two peak times on compound trials were reliably different, these data suggest that a single temporal expectation is not always generated to a compound cue, and open the possibility that the temporal integration seen previously may result from biased responding for only one of the durations. A similar pattern of responding is observed in patients with Parkinson's Disease (PD) when they are asked to reproduce two different durations (Malapani, Rakitin et al. 1998), in that their reproductions are biased towards each other (i.e., the "migration effect"). Data will also be presented assessing the influence of dopaminergic and serotonergic manipulations using this same procedure.

Disclosures: B.J. DeCorte: None. **M.S. Matell:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: David Weil Endowment fund

R01EY13692

Title: Responses of monkey V4 neurons to Glass patterns

Authors: *Y. FU, M. BASSO;
Semel Inst. of Neurosci. and Human Behavior, UCLA, Los Angeles, CA

Abstract: Area V4 of the monkey visual cortex is a midlevel structure in the ventral stream of areas responsible for processing form information. Glass patterns are created by pairing dots in a random array in a characteristic pattern (Glass, 1969); we used translational patterns in which each dot pair had the same orientation. Neurons in areas V1 and V2, which provide input to V4, respond to local cues in Glass patterns and do not signal information about the global form in the stimulus (Smith et al., 2002, 2007). An open question is where in the visual hierarchy of monkey are local cues in Glass patterns converted to global form percepts. As a first step toward understanding this, we measured the responses of 36 V4 neurons in one monkey to translational dynamic Glass patterns (dot size: 0.06 degree; dot separation: 0.1-1.2 degree; dot density: 30-40 dots/degree^2/s; frame rate: 85 Hz) and compared these to responses to oriented Gabor stimuli (SF: 1.1-3.4 c/degree). V4 neurons are tuned for Glass pattern dot-pair orientations in a manner similar to their tuning for Gabor gratings (Spearman's rho=0.54, $p<0.01$). Roughly 60% of V4 neurons have a ≤22.5 degree difference between the optimal orientation for Glass patterns and Gabor stimuli. 80% of the neurons have a difference of ≤45 degrees. However, the average orientation selectivity index (by an index of 0 a cell responds equally to all orientations, and by an index of 1 a cell responds only to a single orientation) of V4 neurons was lower for Glass patterns than for Gabor stimuli (0.12±0.02 versus 0.28±0.03; $p<0.001$). We also measured the response modulation (the difference between the maximum and the minimum of the orientation tuning curve) for Glass pattern and Gabor stimuli. For Glass patterns the response modulation was 8.2±1.2 imp/s whereas for Gabor stimuli the response modulation was 11.6±1.4 imp/s. This difference was significant ($p<0.05$) indicating that neurons in V4 show greater orientation tuning and more robust responses for Gabor than Glass pattern stimuli. Based on these preliminary results, we conclude that area V4, like areas V1 and V2, plays a role in processing local cues in Glass patterns. We hypothesize that the construction of a global orientation percept from Glass patterns occurs downstream of area V4 (Kourtzi et al., 2003; Altmann et al., 2003; Ostwald et al., 2008) or requires information from multiple areas simultaneously (Hedges et al., 2011).

Disclosures: Y. Fu: None. **M. Basso:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH-NIMH R01 MH079511

Title: Spatial rate/phase codes provide landmark-based error correction in a temporal model of theta cells

Authors: *J. MONACO[1], H. T. BLAIR[2], K. ZHANG[1];
[1]Biomed. Engin., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: The spatial firing patterns of place cells in hippocampus and grid cells in entorhinal cortex form a spatial representation that is stable during active navigation but also able to encode changes in external landmarks or environmental structure. One class of model that has been investigated as a possible mechanism for generating these spatial patterns relies on temporal synchronization between theta cells, which fire strongly with the septohippocampal theta rhythm (6-10 Hz) and are found throughout the hippocampal formation, that act as velocity-controlled oscillators. However, a critical problem for these models is that the oscillatory interference patterns that they generate become unstable in the presence of phase noise and errors in self-motion signals. Previous studies have proposed hybridizing temporal models with attractor network models or integrating environmental feedback from sensory cues. Preliminary data from subcortical regions in rats suggest that some theta cells exhibit spatially selective firing similar to hippocampal place fields or entorhinal/subicular boundary fields. These cells also demonstrate a consistent phase relationship across space, relative to ongoing hippocampal theta and to other simultaneously recorded cells, that is correlated with the firing rate at a given location. Inspired by this data, we present a novel synchronization model in which place cells or boundary-vector cells provide a stable, landmark-based excitatory input that drives a rate-to-phase mechanism to generate a population of cells that act as location-controlled oscillators. These cells fire preferentially at theta phases that are specific to a given location, determined by the presence of external landmarks. We show that these location-controlled oscillators provide a stable spatial reference that corrects phase errors in velocity-controlled oscillators, thus preventing the encoded position from drifting with respect to the environment and the actual position of the animal. Thus landmark-based rate/phase correlations in extrahippocampal areas may provide the sensory feedback required by temporal models of neural representations of space.

Disclosures: J. Monaco: None. **H.T. Blair:** None. **K. Zhang:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Support: Research Council of Norway

European Research Council

Title: NMDA receptor regulates phase lock firing of CA1 principal cells during theta oscillation

Authors: M. FALLAHNEZHAD[1,2,3], T. KITANISHI[3], N. KITANISHI[3], *A. TASHIRO[1,2,3]; [1]Warwick-NTU Neurosci. Programme, Sch. of Biol. Sci., Nanyang Technological Univ., Singapore, Singapore; [2]Warwick-NTU Neurosci. Programme, Sch. of Life Sci., Univ. of Warwick, Coventry, United Kingdom; [3]Kavli Inst. for Systems Neurosci., Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: Neuronal firing is modulated by different types of local oscillatory activity. Theta oscillation is one of those found in the mammalian hippocampus during exploratory behavior and REM sleep. Theta oscillation is thought to separate functional states of the network along time and provide time windows for neurons to be involved in different memory processes. It is well-known that principal cells in the hippocampus fire preferentially at a specific phase of theta oscillation, which is a phenomenon termed as theta phase locking. While previous studies suggest that inhibitory input plays a role in theta phase locking, it has not been well investigated how excitatory input contributes to it. In this study, we focused on NMDA receptor, which is a type of ionotropic glutamate receptor and is involved in multiple types of synaptic plasticity, and examined its role in theta phase locking and other firing properties of CA1 principal cells. For this purpose, we devised recombinant adeno-ascociated viral vectors to achieve local RNA interference against NR1 gene, which encodes an essential subunit to form functional NMDA receptor. We achieved virus transduction in a minor portion of CA1 area of rats and performed tetrode-based unit and local field potential recording from the virus-transduced area. This approach allowed us to monitor the activity of NR1-ablated neurons in freely behaving rats. The NR1-ablated neurons showed higher variability in locking phase during theta oscillation indicating that NMDA receptor regulates temporal allocation of neuronal firing along theta oscillation. Thus, NMDA-receptor-dependent synaptic plasticity may contribute to theta phase locking by making neurons more susceptible for firing at a preferred phase. In addition, we found impairment in spatially-modulated firing (place-cell activity), which suggest that temporal and spatial properties of neuronal firing in the CA1 may be mechanistically linked by NMDA receptor.

Disclosures: M. Fallahnezhad: None. **A. Tashiro:** None. **T. Kitanishi:** None. **N. Kitanishi:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: BMBF

MIWF NRW

CoEN

DFG/SFB1089

Title: The neuronal microcircuits of the medial septal nucleus and the diagonal band of Broca

Authors: *H. KANEKO[1], L. SOSULINA[1], F. FUHRMANN[1,2], D. FRIEDRICHS[1], A. GAUCHEL[2], D. JUSTUS[1], S. SCHOCH-MCGOVERN[3], S. REMY[1,2];
[1]German Ctr. for Neurodegenerative Dis. (DZNE), Bonn, Germany; [2]Dept. of Epileptology, [3]Inst. of Neuropathology, Univ. Hosp. of Bonn, Bonn, Germany

Abstract: The medial septal-diagonal band of Broca (MS/DB) plays an important role in hippocampal function by generating theta frequency oscillations (4 Hz-12 Hz). Three main neuron types have been described in this region: GABAergic, cholinergic and glutamatergic neurons, which were characterized electrophysiologically and by the expression of molecular markers. Septohippocampal and intraseptal synaptic connectivity has been demonstrated for all three types. To understand the output of each MS/DB cell type to the hippocampus *in vivo*, it is first necessary to understand the synaptic coupling between these cell types within the MS/DB. Therefore, we investigated the connectivity in the medial septum using whole cell patch clamp recordings and optogenetic stimulation of ChR2-EYFP (H134R) expressing neurons in PV-cre, ChAT-cre and VGluT2-cre mouse lines. We used eYFP expression and immunohistochemistry for cell discrimination. Then a discriminant analysis using the electrophysiological parameters of identified ChR2-EYFP expressing PV+, ChAT+ and VGluT2+ neurons was used in order to discriminate non-eYFP(ChR2) expressing neurons into three groups. In our study, ChR2 expressing neurons could be directly activated by blue light (473 nm) and stimulation evoked reliable action potential firing in each neuron type. The light stimulation also evoked post-synaptic responses in non-eYFP(ChR2) expressing neurons, which were identified by application

of synaptic blockers (glutamatergic: 10 NBQX, 50 D-AP5, GABAergic: 1 SR95531 , 1 CGP52432, cholinergic: 10 Atropine, 0.2 MLA [in μM]). In PV-cre mice, stimulation of PV+ neurons evoked IPSP in 57% of GABAergic, 56% of glutamatergic and 60% of cholinergic neurons, respectively. The predominant number of recorded neurons in VGluT2-cre mice were synaptically coupled and showed either EPSPs or EPSP/IPSP sequences. Only long 10 Hz (20 s) light stimulation evoked a sustained depolarization of MS/DB neurons in ChAT-cre mice. Thus, glutamatergic transmission by VGluT2+ neurons is well suited to drive the activity of downstream cholinergic and GABAergic neurons, while GABAergic neurons provide synchronization and cholinergic neurons modulate the firing rate of MS/DB neurons.

Disclosures: H. Kaneko: None. **L. Sosulina:** None. **F. Fuhrmann:** None. **D. Friedrichs:** None. **A. Gauchel:** None. **D. Justus:** None. **S. Schoch-McGovern:** None. **S. Remy:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: The Japan Society for the Promotion of Science, Grants-in-Aid for Young Scientists (B) 23730712

Title: Effect of an intra-dorsal striatum injection of a muscarinic acetylcholine m1 receptors blocker, pirenzepin, on memorization of duration in rats

Authors: *T. HATA;
Psychology, Doshisha Univ., Kyotanabe, Japan

Abstract: The Striatal Beat Frequency (SBF) model (Matell & Meck, 2004) of interval timing proposes that synaptic plasticity in the dorsal striatum is important for memorization of duration. We have previously reported that an intra-dorsal striatum injection of a NMDA-type glutamate receptors blocker, AP-5, impaired the memorization of duration. Not only NMDA receptors, but also the muscarinic acetylcholine (mACh) receptors are reported to be important for representative synaptic plasticity, long term potentiation, in the dorsal striatum (Calabresi et al., 1999). So, we expected that muscarinic receptors antagonist will also impair the memorization of duration. Following enough number of trainings in peak interval 20-s (PI 20) procedure, the required time of the task was shifted from 20-s to 40-s and the training continued for three

sessions ("time-shift sessions"). Before starting each time-shift session, a mACh m1 receptors blocker, pirenzepin (10µg, 0.5µl) or aCSF was injected into the dorsal striatum. We found that the response rate functions was flattened in the time-shift sessions and the DI value, an index of the precision of timing, was lower in the pirenzepin group than the aCSF group. The shift of peak time toward 40-s in the time-shift sessions was not significantly delayed by pirenzepin treatment. These findings suggest that the memorization of duration was not impaired, but precision of timing was mainly impaired in this condition.

Disclosures: T. Hata: None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: CRCNS US-German collaboration in computational neuroscience (01GQ1110)

Title: A single cell type in the medial septum controls initiation and intensity of movement and matches movement intensity to the hippocampal theta rhythm

Authors: *D. JUSTUS[1], F. FUHRMANN[2], H. KANEKO[1], L. SOSULINA[1], T. BEUTEL[1], D. FRIEDRICHS[1], S. SCHOCH-MCGOVERN[3], M. FUHRMANN[1], S. REMY[1];
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Abstract: A central question in neuroscience is how the intensity of voluntary motor performance is translated into matched synchronized oscillatory activity of brain circuits during motor activity. The medial septum plays a central role in integration of motor programming by relaying ascending input from the pontine-hypothalamo-septal pathway to the hippocampal formation. It is a primary extrinsic regulator of theta rhythm that orchestrates the firing of hippocampal neurons at theta frequencies. In this study we used cell type-specific optogenetic stimulation to discharge either medial septal glutamatergic neurons (VGLUT2+), cholinergic neurons (CHAT+) or inhibitory interneurons (PV+) while the mice performed on a spherical treadmill. We found that rhythmic optogenetic activation of either VGLUT2+ or PV+ neurons effectively entrained the field response of the CA1 hippocampal network to the stimulation frequency. Interestingly, stimulation of VGLUT2+ had a striking behavioral effect on the mice.

It reliably induced forward movement after a delay of several hundreds of milliseconds. Stimulation durations below 1s (4 pulses at 6 Hz) were sufficient to induce movement. Moreover, the speed of the locomotion could be predicted by the frequency of VGLUT2+ cell firing preceding motor activity and depended on the number of activated VGLUT2+ medial septal neurons. Using two-photon Ca2+ imaging of the CA1 subfield (≈1250 imaged neurons, n=4 mice), we observed that the discharge rate of VGLUT2+ neurons in the theta range (3 Hz, 6 Hz, 9 Hz, 12 Hz) positively correlated with the number of CA1 Ca2+ transients. Whole-cell recorded CA1 pyramidal neurons were reliably depolarized and showed synchronized action potential output during stimulation. Thus, the activity of VGLUT2+ neurons may be well suited to match hippocampal theta frequency to the intensity of movement and the recruitment of CA1 neurons. Medial septal stimulation of PV+ neurons (20 s at 10 Hz) led to a reduction in CA1 Ca2+ transients (≈720 imaged neurons, n=3 mice) and reduced action potential output, while stimulation of ChAT+ neurons mildly increased Ca2+ transients (≈850 imaged neurons, n=3 mice) and action potential output. Neither the stimulation of PV+ nor of ChAT+ neurons showed an effect on mice behavior. Glutamatergic blockade locally within the medial septum showed only a modulatory effect on the movement, confirming that the effect on movement was mediated by septofugal projections of VGLUT2+ neurons. However, glutamatergic blockade strongly diminished the optogenetically evoked hippocampal theta oscillations demonstrating the importance of glutamatergic connectivity within the medial septum.

Disclosures: D. Justus: None. **F. Fuhrmann:** None. **H. Kaneko:** None. **L. Sosulina:** None. **T. Beutel:** None. **D. Friedrichs:** None. **S. Schoch-McGovern:** None. **M. Fuhrmann:** None. **S. Remy:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 752.12/UU30

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS0922075

Title: Lesions of the retrosplenial cortex impair temporal learning

Authors: *T. P. TODD, H. C. MEYER, D. J. BUCCI;
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Abstract: Episodic memory involves binding individual objects or events together in place and time (Cohen & Eichenbaum, 1993). The hippocampus has a central role in episodic memory (Tulving & Markowitcsh, 1998), and depends critically on processed sensory information provided by two distinct cortical circuits. One circuit provides the hippocampus with information about the specific object or event, while the other circuit (so called 'where' pathway) provides information regarding the environment, or context, in which the object/event occurred. The exact functions and individual contributions of the components of these circuits remains unresolved, however, growing evidence suggests that the retrosplenial cortex (RSC) has an essential role in forming associations between sensory cues that form the context (e.g., Robinson et al., 2011; 2012). In the present study, we tested the hypothesis that the role of the RSC in processing environmental cues extends beyond physical stimuli by testing the role of the RSC in "when" learning, using a temporal discrimination learning paradigm (see Bouton & Hendrix, 2011). Rats received either sham-lesions (n = 8) or electrolytic lesions (n = 8) of the RSC along the entire rostro-caudal extent. Following recovery, rats were trained in a temporal discrimination procedure, in which daily training sessions consisted of eight presentations of a 10-s tone. For all rats, the tone was paired with food 4 times, and presented alone 4 times. However, the time between tone presentations was a signal for when the tone would be reinforced. When the tone was presented after 16 minutes (Long), it was reinforced, but when the tone was presented after 4 minutes (Short) it was not reinforced. Successful discrimination in this procedure requires rats to respond more to the tone after Long trials compared to Short trials. Results from this experiment suggest that RSC lesions impair this form of discrimination learning. Indeed, after 10 daily session, Sham operated controls demonstrated a clear discrimination; responding more to the tone on Long trials relative to short trials. In contrast, RSC-lesioned rats responded non-differentially to both trial types. Eventually, with continued training, the RSC-lesioned rats were capable of discriminating trial types. Overall, these data are among the first to demonstrate a clear role for the RSC in temporal learning, and further support the critical contribution of RSC to processing 'where' and 'when' information.

Disclosures: **T.P. Todd:** None. **H.C. Meyer:** None. **D.J. Bucci:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

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Program#/Poster#: 752.13/UU31

Topic: F.02. Animal Cognition and Behavior

Support: BMRC Singapore

Title: Identification of neural circuitry for anticipation by whole-brain calcium imaging in larval zebrafish

Authors: R. K. CHENG[1], *T. B. PENNEY[2], S. JESUTHASAN[1,4,3];
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Abstract: The brain is able to extract temporal information from the environment to make predictions about the future, which allows animals to anticipate critical events with a precision of seconds. To identify the underlying neural circuits, we imaged zebrafish larvae (5-9 days-post-fertilization, dpf) expressing genetically encoded calcium indicators (GECIs) throughout the brain. Two-photon laser microscopy, using resonant scanning and fast focusing to enable volume imaging, was carried out on fish that were shown a sequence of 4 pulses of single-color light in a dark background. Each pulse of the stimulus was 20-sec long and followed by a fixed 20-sec inter-stimulus-interval (ISI). From the fluorescence change as a function of time, k-means clustering identified several clusters of cells that responded strongly at or near the time window that an omitted periodic stimulus was scheduled to occur. This type of omitted stimulus response (OSR) was previously reported in rodent and amphibian retinal ganglion cells (RGCs), but only for a very short time scale (msec). In contrast, the zebrafish OSR was observed even with a 20-sec ISI, suggesting the involvement of interval timing. The OSR cells were predominantly located in the habenula along with a few other cells scattered in the anterior and dorsal pallium. Further tests showed that the OSR was triggered regardless of the wavelength being used (red, blue or UV) for the periodic stimulus, and was seen with different ISIs ranging from 6- to 20-sec. In conclusion, the "long" OSR in the current study provides a strong indication that a larval zebrafish, as young as 5 dpf, is capable of perceiving and anticipating a sensory stimulus based on its temporal pattern in the range of seconds. Further imaging should enable identification of a complete circuit mediating timing and time perception in a vertebrate.

Disclosures: R.K. Cheng: None. **T.B. Penney:** None. **S. Jesuthasan:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH099706

NIH Grant DA029330

Title: Two-stage model for concurrent interval timing in non-human primates

Authors: *M. R. KLEINMAN, H. SOHN, D. LEE;
Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Complex behaviors often require estimation of multiple temporal intervals simultaneously, whether to plan future actions or allocate attention when necessary. A pedestrian crossing a busy street must estimate the time it will take to walk across the street, the time for cars to reach the pedestrian, when the ”walk” sign will change, and more. To gain insights into the neurobiological substrates of such concurrent interval timing, we developed a novel oculomotor timing task, in which rhesus monkeys were required to time two independent temporal intervals. The animal repeatedly received a small juice reward according to a Poisson process for maintaining its fixation on a central fixation target, which remained visible throughout the entire session. This introduced a fixed opportunity cost to gaze shifts away from the central fixation target. In addition, two peripheral targets were illuminated along the horizontal meridian with their positions (left or right) and onset asynchrony (0-15s) between them randomized across trials. The color of each peripheral target indicated the fixed interval (FI; 8 or 16s) between the target onset and the time when it was baited with reward. Saccade to a baited target resulted in large juice reward and the extinguishment of that target. Premature saccades generated to unbaited targets before the expiration of their FI were unrewarded, but did not extinguish the target, allowing the animal to produce additional saccades to it. We found that the time of first saccade reliably differed for the 8s and 16s intervals, while subsequent saccades followed with shorter latencies similar for both intervals. Modeling the timing of saccades supported a two-state model, with first saccades generated by one process fit well with a Weibull distribution (mean ~ 10 and 14.5s for 8 and 16s FI, respectively) and a probabilistic transition for later saccades to a second process fit well with an inverse gamma distribution with much shorter latency (mode ~ 1s). Behavior in concurrent interval trials, in terms of distributions of saccade times, obtained reward rate, and fitted model parameters, was similar to single interval trials, except for trials with relatively large onset asynchrony, which systematically decreased the latencies of the first saccades to the later target. Therefore, concurrent timing per se had little effect on timing estimation or precision, demonstrating a clear capacity for simultaneous interval timing in non-human primates.

Disclosures: M.R. Kleinman: None. **H. Sohn:** None. **D. Lee:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 752.15/UU33

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS 1026046

Title: How does temporal spacing influence hippocampal ensemble representations for two contexts?

Authors: *T. E. WHITE, T. CHANG, V. LIAO, J. CZERNIAWSKI, J. F. GUZOWSKI; Univ. of California, Irvine, Irvine, CA

Abstract: The formation of specific contextual memories may be facilitated by the activation of distinct hippocampal neuronal ensembles. The interval separating two experiences is one possible factor that could influence the formation of distinct neural representations. This study investigated how timing of exposures to two contexts influences neural ensemble representation in the dorsal hippocampus. Young adult male Sprague-Dawley rats were exposed to environments A and B for two days with different training groups defined by the interval between exposures to each environment (immediate/no delay, 20 min, 50 min, or 24 hr). Notably, the 24 hr delay group was exposed to context A twice on day 1 and then exposed twice to context B on day 2, with an interval of 50 min between exposures within each day. At the end of training, all groups had spent an equivalent amount of time in both A and B environments. On the third day all subjects were given an identical final presentation to both environments lasting 5 minutes total (2.5 min in A, 2.5 min in B) then sacrificed immediately to detect expression of the activity-dependent immediate-early gene Arc. Arc pre-mRNA is maximally induced 5 minutes after neuronal activity and allows for detection of the total cellular ensemble activated by the two behavioral experiences. Arc transcription is induced in a discrete ensemble of hippocampal neurons by exposure to one environment. By contrast, two behavioral experiences within a 5 min period can be expected to activate two ensembles that may vary with respect to degree of overlap. Thus, we can infer the extent to which different training protocols led to more discrete neural representations by using qRT-PCR to measure intronic Arc expression. For instance, if these two neural representations are nearly independent with minimal overlap, then total Arc expression will be high. Conversely, if representations have a high degree of overlap, then total Arc expression will be low. While all trained groups exhibited an increase in Arc gene

expression compared to untrained home cage controls, subjects receiving a 24 hr delay between exposures to A and B had significantly higher levels of Arc gene expression compared to other trained groups. These data suggest that the 24 hr delay group formed more discrete neural representations for contexts A and B than the other trained groups. Ongoing experiments using fluorescence *in situ* hybridization will confirm and extend the qRT-PCR results by examining Arc expression in different hippocampal subfields.

Disclosures: T.E. White: None. **T. Chang:** None. **V. Liao:** None. **J. Czerniawski:** None. **J.F. Guzowski:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH65561

NIH Grant MH73057

Title: Green tea supplements increase resilience to emotional distractions in an interval timing task

Authors: *A. R. MATTHEWS, B. K. BOGOEV, B. Z. YANG, M. BUHUSI, C. V. BUHUSI; Dept. of Psychology, Utah State Univ., Logan, UT

Abstract: Green tea extracts have been shown to increase activity in prefrontal cortex, with possible beneficial effects on memory and attentional performance. To further probe the impact of green tea supplements on memory and attention, we examined the effect of a green tea flavonoid-rich extract on interval timing with emotional distracters. The presentation of task-irrelevant emotional distracters during a timing task has been shown to delay interval timing behavior. In a previous study in our lab we found that local infusion of antidepressants into medial prefrontal cortex (homologuos to the human frontal lobe) decreases the timing delay after emotional distracters (Matthews et al. Frontiers in Integrative Neuroscience 2012). Therefore, we hypothesized that exposure to green tea flavonoid-rich extracts would decrease the timing delay after emotional distracters. In our study, male C57BL6 mice were divided into three groups: Mice in the SHOCK and SHOCK-GTE conditions received pairings of the distracter stimulus

with a mild footshock, while mice in the NO-SHOCK condition did not. For the following two weeks, mice in the SHOCK-GTE condition received free access to a 2% solution of green tea extract (Polyphenon60, Sigma) in water, while the other mice received free access to tap water in their home cages. During this interval, all mice were trained in a peak-interval procedure. Mice were then tested by unexpectedly presenting the distracter stimulus during the peak-interval test. Mice in the NO-SHOCK condition delayed minimally after the distracter, while mice in the SHOCK condition delayed reliably more, consistent with previous findings in our lab. Instead, mice in the SHOCK-GTE condition showed reliably less delay than SHOCK mice, not different from the NO-SHOCK mice, suggesting that consumption of the green tea extract increased resilience to emotionally-charged distracters. Given that green tea appears to have beneficial effects on attention and working memory in our paradigm, we also examined brain activation in brain regions critical for this paradigm. To determine brain activation levels, c-fos staining was applied to brain sections of the frontal cortex, striatum, and amygdala. Analyses suggested differential levels of activation in the frontal cortex between mice receiving green tea extract and those which received only water. Taken together, these findings suggest that green tea flavonoid-rich extract have beneficial effects on working memory following emotional distraction by differentially activating critical brain regions involved in the interval timing task.

Disclosures: **A.R. Matthews:** None. **B.K. Bogoev:** None. **M. Buhusi:** None. **C.V. Buhusi:** None. **B.Z. Yang:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 752.17/UU35

Topic: F.02. Animal Cognition and Behavior

Support: DFG YO 177/4-1

Title: A hybrid of intracellular and synaptic mechanisms supports a robust *in vivo*-like persistent firing

Authors: ***M. YOSHIDA**[1], A. JOCHEMS[2];
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Abstract: Persistent neural firing is believed to support short-term information retention in the brain. Prevailing hypotheses make use of the recurrent synaptic connectivity to support persistent

firing. However, this mechanism suffers from a lack of robustness. On the other hand, persistent firing can be supported by an intrinsic cellular mechanism in multiple areas in the brain under cholinergic activation, through the calcium activated non-specific cationic (CAN/TRPC) current. Therefore, both the synaptic and the intrinsic cellular (CAN/TRPC current) mechanisms may support persistent firing during memory tasks *in vivo*. However, the consequences of having both the intrinsic and the synaptic mechanisms on persistent firing remain largely unknown. In this study, we investigated whether a neural network model having these two mechanisms (a hybrid model) has advantages over a conventional recurrent network based model, using computer simulations with Hodgkin-Huxley style neuron models. Our results suggest that the hybrid model supports persistent firing within a physiological frequency range over a wide range of different parameters, dramatically reducing parameter-sensitivity issues generally recognized in pure network based models. In addition, persistent firing in the hybrid model was substantially more robust against distracting inputs, could coexist with theta frequency oscillations, and supported pattern completion. These results suggest that the cholinergic system may support persistent firing *in vivo* through CAN/TRPC current.

Disclosures: M. Yoshida: None. **A. Jochems:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant 090813

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Title: Synergy and redundancy in timescale dependent multiplex networks of hippocampal neurons

Authors: *N. TIMME[1], S. ITO[3], M. MYROSHNYCHENKO[2], F.-C. YEH[1], E. HIOLSKI[4], A. M. LITKE[3], J. M. BEGGS[1];
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Physics, [4]Dept. of Microbiology & Envrn. Toxicology, Univ. of California at Santa Cruz, Santa Cruz, CA

Abstract: Understanding the types of computations small groups of neurons perform is of great importance in neuroscience. To investigate these computations, we measured the synergistic and redundant information provided by the spiking activity of pairs of neurons about a third neuron. Generally speaking, synergistic interactions indicate the presence of complex computations, while redundant interactions indicate the retention of common information. In previous work, we found that effective network topology among these neurons was time scale dependent (i.e. the neurons formed temporally dependent multiplex networks) [1]. Therefore, we chose to measure synergy and redundancy at 10 discrete time scales ranging from milliseconds to seconds. To obtain neuron spiking data, we recorded from thousands of neurons across 35 hippocampal slice cultures using a high density 512-electrode array with 60 μm inter-electrode spacing and 50 μs temporal resolution. To the best of our knowledge, this preparation and recording method represents a combination of number of recorded neurons and temporal and spatial recording resolutions that is not currently available in any *in vivo* system. We used transfer entropy (TE) [2] - an information theoretic quantity that can be used to measure linear and nonlinear interactions - to detect significant effective connections between neurons at multiple time scales. Then, we used the partial information decomposition (PID) [3] - a newly developed multivariate information measure that allows for the measurement of simultaneous synergistic and redundant interactions - to measure the synergy and redundancy between groups of connected neurons. We found that neurons with many connections (so called "hubs") transmitted more synergy and redundancy at short time scales than neurons with few connections. To our surprise, we found that network module membership and physical distance between neurons did not affect synergy or redundancy. 1. Timme N, Ito S, Myroshnychenko M, Yeh FC, Hiolski E, et al. Transfer entropy reveals time scale dependent networks in hippocampal and cortical cultures; 2013; Indianapolis, IN. 2. Schreiber T (2000) Measuring information transfer. Physical Review Letters 85: 461-464. 3. Williams PL, Beer RD (2010) Decomposing multivariate information. arXiv: 1004.2515v1.

Disclosures: **N. Timme:** None. **S. Ito:** None. **M. Myroshnychenko:** None. **F. Yeh:** None. **E. Hiolski:** None. **A.M. Litke:** None. **J.M. Beggs:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: JSPS KAKENHI (24223004, 24243069)

Title: Hippocampal-prefrontal coordination is involved in recall of learned sequences in rats

Authors: *S. ISHINO[1], S. TAKAHASHI[2], Y. SAKURAI[1];
[1]Dept. of psychology, Grad. Sch. of Letters, Kyoto Univ., Kyoto/Kyoto, Japan; [2]Neural Circuitry, Brain Sci., Doshisha Univ., Kyoto/Kyoto, Japan

Abstract: Information process of sequential events is essential to predict future events and to act according to a plan. Some studies suggested that coherent theta (4-12Hz) and slow gamma (30-60Hz) oscillations in the hippocampal-prefrontal network in local field potential (LFP) and phase-locking of prefrontal cortex (PFC) spikes to hippocampal theta oscillation play critical roles in working memory (Jones & Wilson,2005; Sigurdsson et al.,2010), which could be a basic function for information process of sequence. In addition, some studies suggest directionality of functional connectivity that the hippocampus leads PFC (Adhikari et al.,2010; Jones & Wilson,2005; Siapas et al.,2005). However, little is known about the relation between these phenomena and recall of sequence memory. Thus, we are recording spikes and LFPs from the hippocampus and PFC simultaneously while the rats are performing the cued serial reaction time task (Ishino & Sakurai, 2014), in which three successive nose-poke responses to successively presented different holes are required. In the task, only two sequences of holes are fixed and learned in advance (predictable sequences) and the other sequences of holes are not fixed (unpredictable sequences). We are analyzing coherence of LFP oscillations, spike-LFP phase locking and directionality of functional connectivity between the hippocampus and PFC, and will report coordinated activity of hippocampal-prefrontal network related to the recall of learned sequences of stimuli.

Disclosures: S. Ishino: None. **Y. Sakurai:** None. **S. Takahashi:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Mercator Stiftung

SFB 874

Title: The processing of space, time, and episodes along the proximo-distal axis of CA1 and CA3

Authors: *Z. BEER[1], K. RENTZING[2], M. SAUVAGE[3];
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Abstract: Episodic memory involves the integration of 'when' and 'where' a specific event ('what') occurred. It is consensus that the hippocampus supports episodic memory in humans and animals. Recent work suggests a functional division along the proximo-distal axis of the hippocampal subfields CA1 and CA3 with the proximal part of CA3 (close to the dentate gyrus) and the distal part of CA1 (close to the subiculum). However, it remains unknown whether this possible functional segregation holds when memory the different information types of episodic memory are segregated in the form of 'what-when' and 'what-where', versus the processing of 'what-when-where'. To address this issue, the current study used molecular imaging of the immediate-early gene Arc, commonly used as a signal of neuronal activation, in combination with an episodic-like memory task in which temporal, spatial, and episodic-like memory can be dissociated. Our results show that the temporal memory for objects is solely supported by the distal part of CA1, while in striking contrast the memory for object locations involved all hippocampal sub-regions, but distal CA1 the least. In addition, distal CA3, which contains the highest level of recurrent connections (suggested to sustain pattern completion), was more activated during episodic-like memory than all other hippocampal sub-regions. In summary, these data bring further evidence of a functional segregation along the proximo-distal axis of CA3 and CA1, based on the processing of temporal, spatial, or episodic-like memory. Furthermore, the results revealed several correlations between memory performance and Arc expression, further strengthening the link between Arc, learning, and memory.

Disclosures: Z. Beer: None. **K. Rentzing:** None. **M. Sauvage:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH-073610 to DP

Title: Both dentate spikes and sharp wave ripples contribute to the cortico-hippocampal conversation

Authors: *D. B. HEADLEY, D. PARE;
Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Cortico-hippocampal interactions are thought to underlie numerous cognitive processes. Of particular importance are synchronous population events in the hippocampus, which have been shown to reflect the content of past experiences, along with predicting task acquisition and performance. This has been extensively investigated at the traditional output side of the hippocampal trisynaptic loop, in CA1, which exhibits occasional transient synchronized population bursts, known as sharp wave ripples. In addition, the primary input region of the hippocampus, the dentate gyrus, also produces synchronous population activity. This manifests as large positive local field potentials, referred to as dentate spikes, that have received little attention in the literature. We examined the relationship between both dentate spikes and sharp wave ripples with unit activity throughout the neocortex. Experiments were conducted under a variety of conditions, across natural sleep/wake cycles, and urethane anesthesia. Tetrodes were implanted in temporal and occipital cortical regions, perirhinal, lateral entorhinal, and prefrontal cortex, along with either tetrode bundles or silicon probes in dorsal hippocampus. We found that the incidence of dentate spikes and sharp wave ripples were comparably affected by behavioral state, with most occurring during SWS, fewer during waking, and virtually none in REM. Also similar between them was their correlation with neocortical unit activity. The strongest effects were present during slow-wave sleep, the weakest during REM, and waking was intermediate. Surprisingly, even sensory neocortical regions displayed substantial coordination with dentate spikes and sharp wave ripples. Increases in cortical slow-wave activity enhanced these effects. In addition, urethane anesthesia greatly enhanced cortico-hippocampal interactions. Altogether, our results underscore the importance of exploring both dentate spikes and sharp wave ripples in future investigations of the behavioral consequences of cortico-hippocampal interactions.

Disclosures: D.B. Headley: None. **D. Pare:** None.

Poster

752. Cortical and Hippocampal Circuits: Timing and Temporal Processing II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 752.22/UU40

Topic: F.02. Animal Cognition and Behavior

Title: Theta-privileged interaction of nucleus reuniens and hippocampus

Authors: *P. P. QUILICHINI, L. NALLET KHOSROFIAN, A. GHESTEM, C. BERNARD; Inst. de Neurosciences des Systèmes, INSERM U1106 INS, Marseille, France

Abstract: Recent data suggest the involvement of the nucleus reuniens (NR), located in the ventral midline of the thalamus, on cognitive processes. It shares reciprocal connections with the medial prefrontal cortex and the hippocampus, and would represent a nodal hub to influence prefrontal-hippocampal interactions. Altought well described on the anatomical level, little is known at the electrophysiological level. In this study, we describe the functional interaction between NR and the hippocampal formation (Hpc) *in vivo* in anesthetized rats in two different brain-states, characterized by theta- and slow oscillations. During theta brain-state, theta oscillations were prominent in Hpc but not in NR. They weakly entrained RN neurons. However during this brain-state, NR displayed a strong 7-10 Hz oscillation, which entrained NR neurons, and much less the Hpc neurons. NR activity was coherent with Hpc in a region-specific fashion. This 7-10 Hz oscillatory pattern was comodulated with theta oscillations in SLM only, where the NR afferences project to. The 7-10 Hz oscillation was not present in NR during slow oscillations brain-state, its neurons strongly firing in a bursting mode during the UP state. Altogether, these results indicate a theta-privileged interaction of NR and Hpc, in which NR inputs may act as a gating operator for other inputs in SLM, like those originating from the entorhinal cortex.

Disclosures: P.P. Quilichini: None. **L. Nallet Khosrofian:** None. **A. Ghestem:** None. **C. Bernard:** None.

Poster

753. Prefrontal and Striatal Systems II

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PICT 2012-1519

PIP 112 201101 01054

UBACYT 200 20 100 100 978.

Title: VTA-dependent regulation of neuronal synchrony and entropy in the PFC emerges along trajectories of balanced excitation-inhibition

Authors: C. J. MININNI[1], *B. S. ZANUTTO[2], S. E. LEW[3];
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Abstract: The Prefrontal Cortex (PFC) and Ventral Tegmental Area (VTA) are key brain regions for understanding goal directed behavior. The PFC is proposed to be a decision making structure, integrating sensory information and initiating behavioral responses, while the VTA is known to modulate changes in synaptic efficacy and neural population dynamics in the PFC, through its direct VTA-PFC projections, both dopaminergic and GABAergic In the present work, 4 Long Evans rats were trained in a GO/NOGO sound discrimination task under a head-restrained paradigm. We recorded neural activity in the PFC and the VTA simultaneously during the discrimination task. A pool of 248 single cells (95 from PFC and 153 from VTA) were selected for analysis. While in GO trials, neurons from PFC and VTA steadily increased their firing rate during tone presentation, in the NOGO trials a transient increase was observed, followed by a return to baseline levels. On the other hand, pairwise correlations between PFC neurons changed during the decision period. During GO trials correlations increased significantly, whereas in NOGO trials correlations decreased. Surprisingly, along with the increase in correlation values, pairwise entropy also increased. We show that this singular relationship between correlation and entropy can be explained with a balanced excitation-inhibition model of PFC, in which signal and noise correlation are parameterized. Increasing values of both correlation and entropy could be achieved by reducing signal correlation and rising noise correlation proportionally. The simultaneously recorded VTA activity suggests that the VTA firing could modulate the synaptic conductances among the PFC neurons, thus changing the balance between signal and noise correlation and driving the observed correlation/entropy profile. Taken together, our results suggest that VTA activity drives prefrontal correlations and entropy along trajectories constrained to the balanced excitation-inhibition regime.

Disclosures: C.J. Mininni: None. **B.S. Zanutto:** None. **S.E. Lew:** None.

Poster

753. Prefrontal and Striatal Systems II

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Support: Columbia University Summer Undergraduate Research Fellowship awarded to MS

NIMH Grant RO1MH068073 awarded to PDB

Title: Dendritic morphology in dorsal striatum changes as a result of habit-related context exposure

Authors: *M. SVOBODA[1], A. L. SASSON[2], M. SHEGDA[1], J. C. HORVITZ[3], E. H. SIMPSON[4], K. T. TAYLOR[1], P. D. BALSAM[2,1];
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Abstract: Previous studies have implicated the dorsolateral striatum (DLS) and dorsomedial striatum (DMS) in habit vs. goal directed behavior, respectively. Previous work in our lab has suggested a distinct pattern of gene expression differences in DLS vs. DMS linked to training that result in learned habits, with many of the gene expression changes in neural growth pathways. We therefore hypothesized that behavioral habit formation would be accompanied by differential morphological changes in DMS vs DLS. Separate groups of rats were trained to press a lever on a variable interval 30 s schedule (VI-30) for 3 days (ShortTrain; n=6) or 25 days (LongTrain; n=6). An additional group (LongExposure; n=6) received 25 days of exposure to the test chamber with non-contingent reinforcer delivery yoked to the LongTrain group. All rats were sacrificed 24 hours after the final VI-30 training session. The brains were immediately extracted and golgi stained brains were sectioned to 150 microns, imaged by light microscopy, and traced using Neurolucida software. Neurons were selected from anterior (+1.6mm relative to bregma) and posterior (+0.48 relative to bregma) regions of the striatum. Two neurons from each DLS and DMS at each pole of the striatum were traced. Morphological features analyzed included cumulative dendrite length and spine density. Dendritic complexity was determined by Sholl analysis. Across all subjects, anterior dorsal striatum exhibited higher dendritic complexity as measured by relative number of dendritic nodes and arborization. We found that exposure to reward in the habit-training environment (the LongTrain and LongExposure groups) was accompanied by an increase in dendritic length, especially in anterior DMS. Additionally, exposure to the training environment resulted in a lower rate of decay of dendritic complexity, evidenced by a lower Sholl regression coefficient, in anterior dorsal striatum. These results indicate that long-term exposure to the reinforced context is sufficient to induce an increase in cumulative dendrite length regardless of whether or not responding is required to receive food reinforcers. The current results suggest that morphological changes in dorsal striatum may play a

key role in establishing the relationship between context and reward, perhaps contributing to the context specificity of habits.

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Poster

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Support: DARPA Repair

Title: Spontaneous dynamics of deep-layer prefrontal cortical populations

Authors: ***A. S. BLAESER**, A. V. NURMIKKO;
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Abstract: The prefrontal cortex depends on local, recurrent network activity to perform many of its major functions. We used calcium imaging (including a novel method of image segmentation and time-series analysis optimized for single-photon imaging) and whole-cell electrophysiology, in acute brain slices, to measure the spontaneous activity of populations of pyramidal neurons in layers 5 and 6 of murine medial prefrontal cortex (mPFC). While most neurons were largely silent under baseline conditions, activity was dominated by a small subpopulation of neurons exhibiting slow (< 1 Hz), rhythmic oscillations. At the network level, cells were generally weakly correlated under baseline conditions, and overall activity was modest. To further explore the state-space of mPFC, we applied several distinct neuropharmacological perturbations (NMDA, clonidine, or picrotoxin) to the slices and studied the resulting transitions in activity. Wash-in of NMDA induced a dramatic uptick in overall activity, and in rhythmic activity and synchronized activity in particular. Agonizing alpha-2a adrenoceptors with clonidine induced moderate reduction in overall activity, but had no clear effect on synchrony or rhythmic activity. Blockade of GABAa receptors by picrotoxin increased the activity and synchrony of the networks and, at high concentrations, resulted in epileptiform discharges. However, picrotoxin had no clear effect on rhythmic firing. These results show that the deep mPFC is capable of exhibiting diverse, spontaneous activity patterns, with structure at both the single-cell and population levels, and reveal some of the cellular and molecular factors underlying rhythmogenesis and synchronization.

Disclosures: **A.S. Blaeser:** None. **A.V. Nurmikko:** None.

Poster

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Title: Dopamine and serotonin transporter function in rat orbitofrontal cortex as contributors to impulsive choice and impulsive action

Authors: *M. DARNA[1], J. J. CHOW[2], J. R. YATES[2], J. S. BECKMANN[2], R. CHARNIGO[3], M. T. BARDO[2], L. P. DWOSKIN[1];
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Abstract: Impulsivity, a multifaceted construct, plays a role in drug abuse vulnerability. Dysregulation of dopamine (DA) and 5-hydroxytryptamine (5-HT) systems in medial prefrontal (mPFC) and orbitofrontal cortex (OFC) have been implicated in impulsivity. Extracellular DA and 5-HT concentrations are dependent on both presynaptic release and uptake via the DA transporter (DAT) and 5-HT transporter (SERT). We hypothesize that DAT and SERT function in mPFC and OFC contributes to individual differences in impulsivity. Across 21 days, rats (n=36) were tested in a counterbalanced order for impulsive choice using a delay discounting task and for impulsive action using a cued go/no-go task. *In vitro* [3H]DA and [3H]5-HT uptake assays determined Km and Vmax for DAT and SERT, respectively, in synaptosomes from mPFC and OFC from each rat. Vmax for SERT in OFC, but not in mPFC, negatively correlated (Pearson correlation $r = -0.581$, $p<0.05$) with mean adjusted delay (MAD) in the delay discounting task. Vmax for DAT in OFC, but not mPFC, was negatively correlated (Pearson correlation $r = -0.54$, $p<0.05$) with extinction responding in the cued go/no-go task. To directly evaluate these relationships, selective SERT and DAT inhibitors (fluoxetine at 0, 15, 50 and 150 pmoles/side, or GBR12909 at 0, 5, 15 and 30 nmoles/side) were microinjected into OFC and effects on impulsive choice and impulsive action determined. Following stabilization of MAD scores in the delay discounting task, fluoxetine microinjected into OFC increased MAD scores (decreased impulsivity) in high impulsive rats compared to saline ($t(18) = 2.44$, $p=0.0254$,$n=6$), but had no effect in low impulsive rats ($t(18) = -0.15$, $p=0.8827$, $n=5$). Following stabilization of extinction responses in the cued go/no-go task, GBR12909 microinjected into OFC had no effect

on extinction responses in either high or low impulsive rats. Thus, inhibition of SERT function in OFC reduced impulsivity only in high impulsive rats, which was consistent with the relationship between decreased SERT function *in vitro* and low impulsive choice. Taken together, the results suggest that 5-HT systems in OFC play a role in mediating impulsive choice, whereas DA systems in OFC do not appear to play a role in impulsive action.

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Poster

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Title: Neurocircuitry and receptor mechanisms underlying the differential sensitivity of prefrontal cognitive processes to psychostimulants

Authors: *R. C. SPENCER[1,2], J. S. SHUMSKY[3], B. D. WATERHOUSE[3], C. W. BERRIDGE[2];
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Abstract: Psychostimulants, including methylphenidate (MPH), are highly effective in treating attention deficit hyperactivity disorder (ADHD). These drugs improve a variety of cognitive/behavioral processes dependent on the prefrontal cortex (PFC) in both ADHD patients and healthy subjects. In humans and rats, systemic administration of MPH improves working memory performance in a narrow inverted-U shaped manner. In contrast, we recently

demonstrated that the cognition-enhancing effects of MPH on sustained attention and attentional set shifting are broader and right-shifted relative to that seen in working memory, similar to that previously described for classroom behavior. Currently, the neurobiology underlying the divergent dose-dependent effects of MPH across cognitive processes is not known. Frontostriatal circuitry is known to support higher cognitive function and is implicated in ADHD. Thus, we first compared the degree to which working memory and sustained attention are dependent on various frontostriatal nodes. Temporary inactivation of the dorsomedial PFC, dorsomedial striatum, or ventromedial striatum impaired performance in both tasks, with inactivation of ventromedial striatum producing particularly profound impairment in sustained attention vs. working memory. Further, MPH action in the dorsomedial PFC, but not dorsomedial or ventromedial striatum, was sufficient for improvement in both tasks. Importantly, the dose-dependent effects of intra-PFC MPH on sustained attention was broader and right-shifted (0.5 μg = maximal improvement) relative to working memory (0.125 μg = maximal improvement), identical to that seen with systemic administration. These results indicate that the differing dose-response curves seen in PFC-dependent processes result from mechanisms intrinsic to the PFC. Prior studies demonstrate that PFC α2 receptors promote working memory while α1 receptors promote attentional set shifting. Consistent with this, we observed that blockade of α2, but not α1, receptors in the PFC prevent the working memory enhancing effects of MPH. In contrast, α1 receptor blockade prevented the sustained attention enhancing actions of MPH. Collectively, these studies indicate that the divergent dose sensitivity to psychostimulants observed across PFC-dependent cognitive processes require differential involvement of α1 and α2 receptors in the PFC. Clinically these results raise the possibility that higher doses that maximally control attention may impair processes important for other domains of academic/social functioning and suggest α1-antagonists as a potential adjunct treatment for ADHD.

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Poster

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Title: Dopaminergic modulation in corticostriatal regions mediating stressor controllability

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Abstract: The medial prefrontal cortex and dorsal striatum form a functional circuit that mediates goal-directed instrumental learning. Previously, the role of this circuit has been almost exclusively studied in appetitive learning paradigms. Recently, it has been suggested that this same circuit is engaged by behavioral control over a stressor, and that activation of this circuit is a necessary mediator of the protective effects that are produced by control. Tonic dopamine (DA) signaling is important for the acquisition of action-outcome associations in appetitive learning, and such learning has been shown to modulate extracellular DA levels within corticostriatal regions. The present series of studies were designed to begin to investigate the role of corticostriatal DA in the stressor controllability phenomena. Rats were exposed to a series of 100 tailshocks that could be terminated by making an operant wheel-turn response on a progressive ratio schedule (controllable shock), while a second group of rats was yoked to the first group and received equivalent tailshocks that could not be terminated (uncontrollable shock). Extracellular DA was measured, using *in vivo* microdialysis, in the medial and lateral striatum (DMS/DLS) during exposure to the tailshock sessions. Uncontrollable tailshock significantly increased extracellular DA compared to baseline in the DMS, but not in the DLS. However, controllable tailshock had no effect on extracellular DA compared to baseline in either the DMS or DLS. Additionally, ongoing studies aim to elucidate the role of DA receptor subtypes within the putative cortical striatal circuit that mediates stressor controllability. D1 and D2 receptor mRNA and protein will be analyzed in separate groups of rats after exposure to 0, 25, or 100 trials of controllable or uncontrollable tailshock.

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Poster

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Title: Neural activity in prelimbic cortex is required selectively for the maintenance of working memory

Authors: *J. WISKERKE[1], J. Y. CHOI[1], H. AKHLAGHPOUR[1], E. AHMED[1], N. K. MEHTA[1], Y. VAN MOURIK[2], T. PATTIJ[2], I. B. WITTEN[1];
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Abstract: The prelimbic cortex (PL) has long been associated with working memory performance in rodents. Lesions and pharmacological inactivations of PL impair performance on a range of working memory tasks. In addition, neurons within PL encode multiple aspects of working memory, including task-related activity during the maintenance period. A long-standing but untested hypothesis is that neural activity within PL is required specifically for the maintenance of working memory, rather than other aspects of the task (e.g. updating working memory with new information, generating motor commands, processing reinforcement). Here we used an optogenetic strategy to transiently inhibit PL pyramidal neurons selectively during defined trial epochs within a spatial working memory task in order to resolve which temporal component of a working memory task requires PL activity. Wild type Long Evans rats were injected bilaterally with either CamKII-eNpHR3.0-eYFP (n=8) or CamKII-eYFP (n=7) into the PL, and bilateral optic fibers were implanted immediately above the injection sites. Subsequently, rats were trained and tested in an operant delayed-non-match-to-position task incorporating trials with 1s, 5s and 10s delays. In the initial experiment, pyramidal neurons were optically inhibited in 20% of trials, with the illumination period spanning the time of trial initiation to the time of the rat's choice (laser trials were randomly interleaved with control no laser trials; light parameters: 532nm, ~5-6mW, continuous light). Analysis of the results of this test showed that optical inhibition caused a significant reduction in response accuracy in the NpHR rats ($p<0.05$, repeated measures ANOVA and Bonferroni post-hoc test), while not affecting behavioral performance in YFP rats. In a second experiment, neuronal activity was inhibited during either 1) the updating phase (presentation of the sample lever), 2) the maintenance phase (delay period), or 3) the motor execution phase (lever choice) of a trial. Again, all conditions were randomly interleaved and optical inhibition occurred on only 20% of trials. Inhibition of PL neuronal activity during the maintenance phase, but not the updating or motor execution phases, impaired working memory performance in a delay-dependent manner, again specifically in NpHR rats. There were significant reductions in choice accuracy during the 5s and 10s delay trials ($p<0.05$, repeated measures ANOVA with Bonferroni post-hoc test). Together, these results demonstrate that PL neural activity is selectively required for the maintenance, but not updating or motor execution, of spatial working memory.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Stochastic behavior in rats and its gating by the anterior cingulate cortex

Authors: ***D. G. TERVO**, M. PROSKURIN, M. MANAKOV, M. KABRA, A. VOLLMER, K. BRANSON, A. KARPOVA;
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Abstract: Behavioral variability allows animals to search for the best course of action and imparts unpredictability useful to defeat competitors and predators. Action selection through stochastic choice would make behavior maximally variable and completely unpredictable, but would be at odds with one of the brain's central operating principles: to use experience to optimize behavioral choice. When their choices are rewarded only if they escape prediction by an electronic competitor, primates, in fact, resort to increasingly complex history-based behavioral patterns, in part based on models of the competitor's prediction algorithm, rather than adopting a fully stochastic strategy. We have demonstrated that although rats also use history- and model-based strategies when faced with similar electronic competitors, they switch to a stochastic-choice mode when challenged with a more sophisticated competitor that cannot be normally defeated by counter-prediction. In this stochastic mode, outcomes associated with an animal's actions are ignored, and the influence of the anterior cingulate cortex (ACC) - a brain region involved in guiding behavior based on an experience-derived model of the environment's governing rules - is suppressed. These findings suggest that when uncertainty about environmental rules persists due to an inadequate internal model, changes in ACC output prevents erroneous beliefs from guiding behavioral decisions thus enabling behavioral variation. We are currently exploring the circuit basis of the switch between strategic and stochastic modes of decision-making.

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Poster

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Support: MH46904

MH74006

Title: Responses of medial prefrontal cortex and medial auditory thalamus during acquisition, expression and extinction of trace eyelid conditioning

Authors: ***K. THAKKAR**[1], S. R. FRIEDRICH[2], L. C. HOFFMANN[2], H. E. HALVERSON[2], M. D. MAUK[2];
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Abstract: During auditory trace eyelid conditioning the medial prefrontal cortex (mPFC) and medial auditory thalamic nuclei (MATN) provide the cerebellum with inputs necessary for learning. During this task, the cerebellum uses MATN input from the tone conditioned stimulus (CS) and tone evoked persistent activity from mPFC to acquire conditioned responses over a few hundred paired trials. Persistent activity extending through the trace interval in mPFC is known to be necessary for such learned responses. Trace eyelid conditioning provides an opportunity to investigate how responses in MATN might contribute to mPFC persistent activity from a naïve state to a learned state and how those changes might inform persistent activity. Single unit activity was collected from 18 tetrode hyperdrive arrays targeting the MATN and mPFC during trace conditioning with a tone CS and mild peri-orbital stimulation (US) that were separated by a 500 ms stimulus-free trace interval. Rabbits received paired, unpaired and extinction sessions to investigate neuronal responses in both areas across acquisition, expression and extinction of trace conditioning. Neurons were isolated with an interactive cluster-cutting program (WinClust). An anatomical pathway from MATN to mPFC was examined with anterograde and retrograde tracers. Paired training revealed persistent activity in MATN and mPFC on the first session of training. Expression of trace conditioned responses produced response-related activity in MATN

and mPFC. Extinction training led to a decrease in persistent activity in both areas and stimulus specificity during re-training using a different frequency tone. Following individual neurons in both areas for multiple sessions across the three training phases revealed changes in persistent activity that corresponded with the state of learning. These results provide important insights into our understanding of the critical circuit for trace eyelid conditioning and the possible origin of persistent activity during learning.

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Poster

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Title: Using connectivity to study homology of orbitofrontal and cingulate cortices in primate and rat

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Abstract: The issue of homologies between rodent and primate brains is still greatly debated, particularly when discussing the cingulate and orbitofrontal cortices. Here, we analyzed anatomical connections of structures with relatively accepted homologies to evaluate whether the rat cortex contains areas that are homologous to regions of the anterior cingulate and orbitofrontal cortices in monkeys. The caudal ventromedial prefrontal cortex (vmPFC, area 25) in the macaque is considered to be homologous to the infralimbic cortex (IL) in rats (e.g., Ongur & Price, 2000; Freedman et al., 2000). Likewise, the striatum, especially, the ventral striatum, and the amygdala are largely homologous across the two species. However, the correspondences between anterior cingulate (areas 24 and 32) and orbitofrontal cortex in the monkey and

prelimbic (PL), dorsal cingulate (Cg1), ventral cingulate (Cg2), and the orbital surface in the rat remain unknown. Using our library of injections of anterograde tracers into macaque and rat brains, as well as the existing literature on cortico-cortical, cortico-striatal, cortico-amygdalar, and amygdalar-cortical projections, we developed side-by-side maps of these connections in both rat and monkey. Preliminary analyses of these data indicate conserved patterns of cortico-striatal projections that facilitate identification of homologous regions. Cortico-striatal projections in both species follow a ventromedial to dorsolateral gradient, with projections to the shell of the nucleus accumbens originating in IL (rat) and vmPFC (monkey) (e.g., Haber et al., 2006; Haber et al., 1995; Mailly et al., 2013; Vertes, 2004). OFC axons terminate dorsomedially to IL/vmPFC terminals. Both monkey and rat exhibit a medial-lateral gradient in OFC projections to the striatum, with medial OFC projecting ventromedially within the striatum to lateral OFC. Of particular importance, there is considerable variation in the topography of PL projections to the striatum, suggesting that it can be divided into dorsal vs ventral and rostral vs caudal subregions. We hypothesize that dorsal PL, along with ventral portions of Cg, may be homologous to the dorsal anterior cingulate cortex (area 24), while ventral portions of PL may be homologous to ventral anterior cingulate cortex (area 32).

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Poster

753. Prefrontal and Striatal Systems II

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Title: Daily exposure to novel environments in young mice improves motor patterns in later life or Daily exposure to novel environments in young mice improves motor patterns with age

Authors: *S. L. HONG[1], S. J. BARTON[2], G. V. REBEC[2];
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Abstract: This study examined the role of novelty in environmental enrichment and its effects on spontaneous motor behavior patterns in aging mice (C57BL/6). We compared the effects of

two isolated-housing conditions, Running Wheel [(IHRW; n = 13; mean age 70.8 +/- 17.9 weeks)] and Empty Cage (IHEC; n = 16; mean age 73.9 +/- 16.3 weeks)], against two enriched environments, Static [(EEST; n = 20; mean age 58.1 +/- 12.3 weeks)] and Dynamic [(EEDY; n = 14; mean age 73.8 +/- 15.6 weeks)]. Mice in the enriched conditions were housed 12 mice per cage. The EEDY group had the location of toys as well as food and water sources changed daily, while placement of these items remained constant for the EEST group. Mice were randomly assigned to one of these four groups at ~4 weeks of age and remained in their respective environments for the next 25 weeks. After which, all mice were housed singly in the empty cage condition for the remainder of the experiment. They were removed monthly for behavioral testing, which included placement in a plus maze for a period of 30 minutes. The probability of performing complex motor behaviors, i.e., turning 90 degrees, was assessed. The EEDY group had the highest probability of turning (0.49±0.1), followed by the EEST (0.47±0.1), IHRW (0.43±0.1), and IHEC (0.42±0.1) groups. One-way ANOVA revealed a significant group effect. Tukey post-hoc pairwise comparisons indicated that the EEST and EEDY groups were significantly more likely to perform the 90-degree turn within the plus maze than the IHEC and IHRW groups. We further measured the degree of age-related decline in turning probability using linear regression. Only the slope for the EEST group was negative and significantly different from zero, indicating that the probability of turning in this group declined as a function of age. Regression slopes for all the remaining groups were zero. The benefits of novelty in EEDY mice are indicated by a greater spontaneous performance of more complex motor behaviors, indicating greater behavioral flexibility that is sustained with advancing age. Our results demonstrate that novelty is a critical factor in environmental enrichment, beyond that of socialization and exercise.

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Title: Topography of projections from the subthalamic nucleus to the prefrontal cortex

Authors: *A. C. BOSTAN[1], R. P. DUM[1], P. L. STRICK[1,2];
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Abstract: The subthalamic nucleus (STN) of the basal ganglia (BG) receives direct inputs from the cerebral cortex and projects back to the cerebral cortex via the major BG output nuclei, the internal segment of the globus pallidus and the substantia nigra pars reticulata. This route constitutes the "hyperdirect pathway" (Nambu et al. '96) and suggests that the STN plays an important role in regulating behavior. Because of the multisynaptic circuitry involved, details of the anatomical organization of the hyperdirect pathway and its significance remain poorly understood. Kelly and Strick ('04) demonstrated that dorsal portions of STN receive and send projections back to primary motor cortex (M1). Furthermore, regions of STN that project to M1 are spatially distinct from those that innervate prefrontal area 46. Here, we used retrograde transneuronal transport of rabies virus (RV) to further explore the organization of STN projections to the prefrontal cortex in cebus monkeys. We injected the N2c strain of RV into the lateral portion of area 9 (9L, n=1) and Pre-dorsal premotor cortex (PrePMd, n=2). We set the survival time to allow retrograde transneuronal transport of RV from the injection sites to 3rd-order neurons in the STN and compared the results with previous RV injections into area 46 (n=1) and M1 (n=1) (Kelly & Strick, '04). Following RV injections into prefrontal areas, we observed dense labeling of 3rd-order neurons primarily in ventral portions of the STN. The regions of STN that target the prefrontal cortex were clearly segregated from the more dorsal regions that target M1. The peak density of 3rd-order neurons that project to each prefrontal area appeared to be located within the region that receives afferents from the same prefrontal area (Haynes & Haber, '13). Specifically, projections to area 9L originate from the most ventral regions of rostral and central STN. Projections to PrePMd and area 46 originate from slightly more dorsal regions of STN, with the projections to PrePMd being located lateral to those targeting area 46. Our results demonstrate that STN projections to the prefrontal cortex are topographically organized. Thus, STN output to the cerebral cortex has substantial specificity, in contrast with the current view that STN output is highly divergent (Parent & Hazrati, '93). This specificity indicates that the hyperdirect pathway linking the BG with the cerebral cortex via the STN is organized in parallel closed loop circuits.

Disclosures: **A.C. Bostan:** None. **R.P. Dum:** None. **P.L. Strick:** None.

Poster

753. Prefrontal and Striatal Systems II

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NIH Grant AA023141

Title: mGluR2/3 signaling in the nucleus accumbens shell restores goal-directed behavior

Authors: *J. M. BARKER, D. LENCH, J. CHANDLER;
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Abstract: While alcohol consumption for most individuals is controlled and goal-directed, some individuals go on to develop inflexible stimulus-mediated habitual drinking which may be exacerbated by exposure to ethanol. Here, we demonstrate that chronic intermittent exposure to ethanol (CIE) using a vapor chamber model can facilitate the formation of ethanol-seeking habits. The regulation of habitual behavior likely requires normal activity in key neural substrates including the nucleus accumbens (NAc). Chronic ethanol exposure has been shown to disrupt NAc glutamate levels (Griffin et al., 2013) and to down-regulate mGluR2 expression in the NAc (Meinhardt, et al., 2013). To determine a causal role for mGluR2 signaling in the facilitation of habitual behaviors, we used both local and systemic manipulations of mGluR2 signaling. It was observed that systemic mGluR2 agonism restored goal-directed reward seeking. Furthermore, local infusions of mGluR2 agonists into the NAc shell were sufficient to rescue habitual reward-seeking, suggesting that the observed loss of mGluR2 signaling in the NAc may drive the development of stimulus-response behavior. Together, these findings indicate that chronic ethanol exposure can promote habitual behaviors, characterized by the loss of sensitivity to changes in the action-outcome relationship. This effect appears to be mediated through dependence-induced alterations in mGluR2 expression in the NAc. Given a growing literature suggesting aberrant glutamate signaling in amygdala subregions, current research is investigating whether mGluR2 downregulation is selective to infralimbic projections to the NAc and whether any changes have similar roles in the expression of habitual ethanol seeking as well as the selective effects of enhancing mGluR2 signaling on infralimbic projection neurons to these regions.

Disclosures: **J.M. Barker:** None. **D. Lench:** None. **J. Chandler:** None.

Poster

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Support: NIH Grant DA011717JRT

NIH Grant DK098994RJD

Title: Optogenetic manipulation of context-dependent resistance to extinction in mice

Authors: ***S. M. KATRANCHA**, S. L. QUICK, B. B. LAND, R. J. DILEONE, J. R. TAYLOR; Yale Univ., New Haven, CT

Abstract: Behavioral momentum theory states that there are two separable aspects of behavior: its ongoing rate and its persistence following disruption. The ongoing rate of responding is controlled by the rate of contingent reinforcement, whereas the resistance to change of behavior depends upon the incentive motivational properties of the stimulus context in which it occurs. Despite repeated demonstrations of the utility of this theory, little is known about the neural circuitry that governs context-dependent resistance to extinction. This study will use the procedural framework of behavioral momentum theory to dissociate the response-reinforcer and stimulus-reinforcer relationships that drive behavior. Mice were trained to press a lever for food in two alternating “rich” and “lean” contexts. The amount of contingent reward was equivalent in each context, but mice received additional free food in the rich context. Thus, the response-reinforcer relationship was equivalent across contexts, but the stimulus-reinforcer relationship was relatively greater in the rich context. As predicted by behavioral momentum theory, all mice exhibited greater resistance to extinction in the rich than in the lean context. Additionally, the rate of extinction was similar in each context, suggesting that the mice learned extinction equivalently. Following extinction tests, the impact of the rich and lean contexts on free feeding was investigated. The amount of food consumed in each context was compared, and no significant difference was observed. We are currently investigating the role of dopamine D2-receptor neurons in the nucleus accumbens in mediating contextual control of operant behavior. Specifically, D2-Cre mice were injected with a Cre-inducible channelrhodopsin, implanted with chronic indwelling optical fibers, and trained in the behavioral procedure as described above. We

will test the effect of optogenetic stimulation of dopamine D2-receptor neurons on resistance to extinction in the rich context. We predict that inhibition of reward circuitry via the optogenetic stimulation of dopamine D2-receptor neurons in the nucleus accumbens during the rich context will block the impact of the incentive motivational properties of the rich context such that there will be no difference in resistance to extinction between contexts. This research will help elucidate the neural circuitry underlying context-dependent resistance to change in a variety of disorders, such as obesity in which individuals experience the drive to continue to eat in rich contexts (i.e. fast-food restaurants) despite becoming satiated.

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Poster

753. Prefrontal and Striatal Systems II

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Support: NIAAA Grant 1K22AA020303-0

NIAAA Grant P20-AA017068

Title: Impaired behavioral flexibility and altered cortical firing in a murine moderate prenatal alcohol exposure model

Authors: *K. L. MARQUARDT[1], R. SIGDEL[2], J. CAVANAGH[2], J. BRIGMAN[2]; [1]Neurosciences, BMSB 145, Univ. of New Mexico, Albuquerque, ; [2]Neurosciences, BMSB 145, Univ. of New Mexico, Albuquerque, NM

Abstract: Surviving in a complex, ever-changing environment relies heavily on cortically mediated executive function behaviors, such as working memory, emotional control and behavioral flexibility. Not surprisingly many neuropsychiatric and behavioral disorders, such as schizophrenia, addiction and autism, include deficits in executive function. Recently attention has been drawn to the safety of low to moderate alcohol consumption during pregnancy. High levels of prenatal alcohol exposure (PAE) have been shown to impair flexible behavior; however impairment of executive functioning from low to moderate doses of PAE, such as those that may model deficits found in Fetal Alcohol Spectrum Disorder (FASD), has not been well

characterized. Adolescents with FASD exhibit maladaptive perseveration, or difficulty in flexibly altering their behavior. A cross-species, well-validated measure for behavioral flexibility is discrimination-reversal learning. The cortico-striatal circuit underlying this behavior has been extensively studied. Associative learning during discrimination is reliant on the dorsal striatum (DS), while optimal reversal is dependent upon the orbitofrontal cortex (OFC), as isolated inactivation of these regions selectively disrupts distinct stages of this paradigm. To investigate the effects of moderate PAE on behavioral flexibility we used a touch screen based visual discrimination-reversal task in conjunction with *in vivo* electrophysiology to measure OFC and DS function in a murine model. Dams were given access to a saccharin sweetened 10% alcohol solution for four hours during the dark cycle, throughout gestation, resulting in blood ethanol concentrations of 80-90 mg/dL daily. PAE mice made significantly more perseverative errors during the choice shifting phase of reversal (<20% correct), but were not impaired on discriminative learning. Timeline analysis shows PAE mice remain in the perseverative phase longer than saccharin (SAC) control mice. *In vivo* electrophysiological recordings of phasic baseline firing suggest a delay of lateral OFC recruitment during choice shifting in PAE mice compared to SAC control mice. Analysis of DS firing allowed for examination of concurrent *in vivo* phasic baseline firing alterations in PAE and SAC animals. Dual region *in vivo* electrophysiology between OFC and DS is being used to analyze local field potential phase shifts between SAC and PAE mice, as well as compare phase lock between regions. Together our data demonstrate an enduring impairment in executive function after moderate PAE that may be the result of improper OFC engagement.

Disclosures: K.L. Marquardt: None. **R. Sigdel:** None. **J. Cavanagh:** None. **J. Brigman:** None.

Poster

753. Prefrontal and Striatal Systems II

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Topic: F.02. Animal Cognition and Behavior

Title: Face processing in the monkey orbitofrontal cortex

Authors: *E. BARAT, G. REYMOND, S. WIRTH, J.-R. DUHAMEL;
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Abstract: Faces are complex stimuli that convey information about an individual's identity and emotional states. This information is critically important for non-verbal communication between primates. Face processing is performed by a dedicated network of regions that includes clustered face patches in temporal cortex[1] and prefrontal cortex[2]. Here we questioned specifically the role of the monkey orbitofrontal cortex (OFC) and hypothesized that it underlies the interpretation of information carried by faces and the selection of appropriate behavioral responses. Indeed, not only do OFC neurons respond to visual stimuli such as faces[3] but they also encode the subjective value of stimuli thanks to rapid stimulus-reinforcer association[4,5]. It has also been shown that OFC encode the motivational value of rewards obtained in a social context, suggesting a role in the assessment of social information[6]. In a behavioral experiment, we evaluated the preference for faces considered as socially distinct (male, female, old or young faces) by recording ocular scanpaths in an exploratory viewing task in 4 monkeys. The analysis of viewing preferences showed that monkeys were sensitive to the identity of the stimuli. Specifically, animals spent significantly less time on faces of older monkeys. In parallel, we recorded face cell activity in one monkey near the lateral orbital sulcus, during a simple fixation task. Neurons were first classified as face selective and their selectivity was further studied using different sets of images including neutral, emotional or socially distinct conspecific faces. Specificity for different facial expressions was also tested using videos of conspecifics. Among 108 visual cells tested, 57 (53%) were face selective and we distinguished several subclasses of face cells according to their responses to specific face categories. Interestingly, the category of stimuli to which neurons responded the least was the same that was behaviorally neglected (i.e. older monkeys). Overall, these findings suggest that face cells in the OFC encodes several subjectively significant attributes of face stimuli such as their social or emotional value and thus participates to valuation of faces in the context of social interactions. 1. Tsao, D. Y.*et al*., *Nat. Neurosci.* **6,** 989–995 (2003). 2. Tsao, D. Y. *et al*.; *Nat. Neurosci.* **11,** 877–879 (2008). 3. Rolls, E. T. *et al*., *Exp. Brain Res. Exp. Hirnforsch. Expérimentation Cérébrale* **170,** 74–87 (2006). 4. Thorpe, S. J. *et al*., *Exp Brain Res* **49,** 93–115 (1983). 5. Tremblay, L. & Schultz, W. *Nature* **398,** 704–708 (1999). 6. Azzi, J. C. B. *et al*., *Proc. Natl. Acad. Sci. U. S. A.* **109,** 2126–2131 (2012).

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Poster

753. Prefrontal and Striatal Systems II

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Topic: F.02. Animal Cognition and Behavior

Support: NIAAA Intramural Research Program

Title: Role of prelimbic cortex and its connections to dorsomedial striatum in rewarded learning

Authors: *C. R. PINARD[1], H. C. BERGSTROM[1], O. BUKALO[1], L. ZWEIFEL[2], A. HOLMES[1];
[1]Lab. of Behavioral and Genomic Neurosci., NIAAA/NIH, Rockville, MD; [2]Dept. of Pharmacol. and Psychiatry & Behavioral Sci., Univ. of Washington, Seattle, WA

Abstract: Current evidence suggests that the prelimbic cortex (PL) and dorsal medial striatum (DMS) play a major role in rewarded learning. PL projections innervate the DMS, possibly providing an integrated network for such learning, but this concept has not been directly tested. Here, we employ *in vivo* optogenetics to examine the learning-related effects of silencing PL neurons or their efferent projections to the DMS in a visual discrimination and reversal paradigm. First, we used an adenoassociated virus (rAAV8/CAG-ArchT-GFP) containing the inhibitory proton-pump opsin archaerhodopsin (ArchT) to silence AAV-ArchT-expressing neurons in the PL in C57BL/6J mice during reversal learning at the beginning of stimulus presentation through a touchscreen response and terminating at reward collection. Our second approach was to specifically target parvalbumin (PV) interneurons in the PL to test how increasing PL output affects learning. Despite representing a relatively small proportion of cells in the PL, PV-positive interneurons exert an outsized influence on the firing and synchronization of neuronal activity and on behavioral functions mediated by PL output (e.g., Courtin et al. 2014). Here, we silenced local PV interneurons after expressing ArchT-DIO AAV in the PL of PV-CRE mice. In a third experiment, we defined the pathway connecting the PL to the DMS. To this end, we infused rAAV5/EF1a-DIO-eArch3.0-eYFP into the PL and a retrograde canine adenovirus (CAV2-Cre) (e.g., Hnasko et al 2004) into the DMS in order to specifically silence PL-DMS projections. Together, these three experiments could further our understanding of the role of the PL in rewarded learning. Research supported by the NIAAA Intramural Research Program.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIAAA Intramural Research Program

Title: Differentiating contributions of dorsolateral and dorsomedial striatum to reward learning

Authors: *H. C. BERGSTROM, C. L. PICKENS, C. R. PINARD, O. BUKALO, L. R. HALLADAY, A. HOLMES;
Lab. of Behavioral and Genomic Neurosci., Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: The dorsal striatum can be functionally subdivided into lateral (DLS) and medial (DMS) zones, based on differences in patterns of cortical innervation and contributions to various forms of reward learning. Current models posit a major role for the DMS in outcome-sensitive behaviors and for the DLS in stimulus-driven performance and habits. Recent evidence suggests that the DLS- and DMS-mediated strategies develop in parallel during discrimination learning to compete for control over performance (e.g., Bradfield & Balleine 2013). Consistent with this hypothesis, we have previously found that mice learning a visual touchscreen discrimination exhibit tandem recruitment of the DLS and DMS. Here, we tested the consequences of silencing either the DLS or DMS during visual discrimination learning in C57BL/6J mice. Green light was bilaterally shined on DLS or DMS neurons expressing an adeno-associated viral construct (rAAV8/CAG-ArchT-GFP; ArchT) to silence neuronal activity beginning at stimulus presentation through a touchscreen response and terminating at reward collection. Silencing DLS neurons during this interval facilitated early discrimination learning, as compared to controls expressing an inactive virus (rAAV8/CAG-GFP). This facilitatory effect was exhibited by reduced errors rates as early as the first session of training. By contrast, silencing DLS neurons during reward collection did not affect performance. These data suggest that the DLS exerts an inhibitory influence over learning early in training, possibly by generating stimulus-elicited responses that initially interfere with more rapidly acquired outcome-based responding. As such, these findings suggest that attenuating outcome-based learning, for example by silencing DMS neurons, might have opposite (i.e., retarding) effects on learning by biasing towards DLS-dependent processing. Addressing these outstanding questions will help further define the contributions of the DLS and DMS to reward learning.

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Poster

753. Prefrontal and Striatal Systems II

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI (24240060)

Title: Projections from the amygdala to subregions of the anterior cingulate cortex in macaque monkeys

Authors: *Y. KIM, H. SAKATA, N. KONOIKE, S. MIYACHI, K. NAKAMURA;
Primate Res. Institute, Kyoto Univ., Inyama Aich, Japan

Abstract: The anterior cingulated cortex (ACC) can be functionally divided into subregions. Pervious studies have suggested that the dorsal ACC is involved in appraisal and expression of emotion whereas the ventral ACC regulates the function of limbic regions in emotional responses. Although these functional differences between these ACC subregions may reflect differences in anatomical input-output pattern of them, details of the input-output patterns of the ACC subregions are still unclear. To address this issue, in the present study we investigated the input patterns from the amygdala to the ACC subregions. We injected neuronal tracers (fluoro ruby, fluoro emerald, lucifer yellow-dextran, cholera toxin B subunit) into different ACC subregions of five adult monkeys. Among them, three injection sites were located in the dorsal ACC subregion, the cingulate gyrus above the genu of the callosal body (area 24). Two injection sites were confined in the subgenual region (the ventral ACC subregion centered at area 25). The brain sections were immuno-stained for the tracers, and the distribution of the retrogradely labeled neurons was examined in the ipsilateral amygdala. The distribution pattern was compared between the dorsal and ventral ACC injections. In both the dorsal and ventral ACC injections, many labeled neurons were found in the basal nucleus of the amygdala, where the large majority of the labeled neurons were located in the intermediate subdivision. In contrast, only few neurons were labeled in the lateral and central nuclei. The major difference in input pattern between the dorsal and ventral ACC subregions was found in the accessory basal nucleus. In the cases of injection into the ventral ACC, a considerable number of neurons were labeled in the accessory basal nucleus, whereas only few neurons were labeled in the nucleus in the cases of injection into the dorsal ACC. Functional differences in regulation of emotional behavior between the dorsal and ventral ACC subregions may be partly due to the difference in anatomical input pattern from the amygdala to these two subregions.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 24240060

Title: Projections from the temporal cortical areas to the subgenual portion of the anterior cingulate cortex in macaque monkeys

Authors: *H. SAKATA, Y. KIM, N. KONOIKE, S. MIYACHI, K. NAKAMURA;
Primate Res. Institute, Kyoto Univ., Inuyama, Aichi, Japan

Abstract: The anterior cingulate cortex (ACC) is implicated in the regulation of emotional behavior, and dysfunction of a neural network including the ACC may bring on mood disorder. Recent imaging studies have emphasized the importance of the subgenual portion of the ACC (subgenual ACC), the portion just ventral to the genu of the corpus callosum. However, details of the anatomical connection of the subgenual ACC are unclear since only a few previous studies have described the connection of the subgenual ACC and therefore their results were inconclusive. To elucidate the anatomical basis for the functions of the subgenual ACC, we examined the layer pattern of the cortical afferents from temporal areas to the subgenual ACC. Neuronal tracers were injected into the subgenual ACC in macaque monkeys. The brain sections were immuno-stained for the tracers, and the distribution of the retrogradely labeled neurons was examined in the ipsilateral temporal areas. In the entorhinal and perirhinal (areas 35 and 36) cortices, labeled neurons were mainly found in the layer V. Interestingly, some labeled neurons were also observed in the superficial layers of confined area 35. In the temporal pole and the parahippocampal cortex, labeled neurons were in both the superficial and deep layers. In both the dorsal and ventral banks of the superior temporal sulcus, we also found labeled neurons in both the superficial and deep layers, but more in the superficial layers. As previously reported, we found fewer neurons in the “sensory-related” inferior and superior temporal convexities. The difference in layer distribution of the labeled neurons among different temporal cortical areas may reflect difference in functional relation of the subgenual ACC to these areas.

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Poster

753. Prefrontal and Striatal Systems II

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Interactions between medial prefrontal cortex and dorsomedial striatum are necessary for working memory span in rats

Authors: *D. A. DAVIES, Q. GREBA, J. C. SELK, J. G. HOWLAND;
Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Working memory is a type of short-term memory for storage and manipulation of information necessary for higher order cognition. The capacity of working memory is often assessed by measuring the "span" or number of stimuli that can be maintained in working memory. Previous research has linked working memory span performance to activation in medial prefrontal cortex (mPFC) and striatum. The present experiments examined whether bilateral mPFC inactivation, bilateral dorsomedial striatum (dmSTR) inactivation, or disconnection of mPFC and dmSTR using unilateral infusions into each structure in opposite hemispheres reduced span using the odor span task (OST) in rats. The OST requires rats to remember an increasing span of different odors to receive food reward. A within subjects design was used with male Long Evans rats (n=8). Rats were food restricted for all experiments and had cannulae implanted bilaterally into mPFC and dmSTR using conventional techniques. After shaping the rats to dig in unscented sand for a food reward, the rats were trained in a delayed non-match to sample procedure using scented cups. After rats reliably performed the delayed non-match to sample procedure, subsequent test days involved adding additional cups with novel scents one at a time until an error occurred. The number of cups that the rat correctly chose before it made an error (not counting the first cup) was recorded as the span for that trial. Microinfusions (0.30 µl; 15 min before testing) of the GABA receptor agonists muscimol and baclofen were used to reversibly inactivate mPFC and/or dmSTR. Similar to previous results from our laboratory, bilateral inactivation of mPFC significantly impaired span. The mean span observed following muscimol and baclofen infusions into the mPFC were 2.67 ± 0.9 odors, while vehicle spans were 7.76 ± 1.3. Similarly, bilateral inactivation of dmSTR significantly reduced span (after inactivation, span = 5.64 ± 1.4 odors; after vehicle, span = 10.95 ± 1.5). Disconnection of mPFC and dmSTR using unilateral infusions of muscimol and baclofen into opposite hemispheres for each structure significantly impaired span (after inactivation, span =

1.69 ± 0.4 odors; after vehicle, span = 6.83 ± 2.0). Latency for the rats to dig into a bowl was not affected by any of the treatments administered. The present results demonstrate a critical role of the direct projection from the mPFC to the dmSTR in performance of the OST in rats.

Disclosures: D.A. Davies: None. **Q. Greba:** None. **J.C. Selk:** None. **J.G. Howland:** None.

Poster

753. Prefrontal and Striatal Systems II

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Topic: F.02. Animal Cognition and Behavior

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Title: Identification of dopamine and norepinephrine receptor transcripts in prefrontal cortical pyramidal cells defined on the basis of projection target

Authors: *H.-D. WANG[1], M. J. M. MURPHY[2], M. S. SIMMONS[4], J. H. MEADOR-WOODRUFF[4], A. Y. DEUTCH[3];
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Abstract: Dystrophic changes in pyramidal cell (PC) dendrites of the prefrontal cortex (PFC) in schizophrenia have been reported by a number of groups. We have previously shown that PFC dopamine (DA) depletion decreases the density of dendritic spines on PFC layer 5 PCs, consistent with the reported decrease in the dopaminergic innervation of the PFC and the decrease in dendritic spine density of PFC PCs in schizophrenia. In addition, we have found that specific groups of layer 5 PCs are susceptible to dopamine denervation-induced spine loss; these different populations of PCs can be identified based on their projection target. Although DA denervation-induced dendritic spine loss is restricted to vulnerable PCs that innervate specific subcortical projection targets, the mechanism through which this occurs is not clear. In an effort to clarify the receptors that subserve this dendritic remodeling, we identified catecholamine receptor transcripts in different populations of PFC cells that innervate various targets. Animals were injected with fluorescent latex microspheres (beads) and one week later PFC neurons expressing the beads isolated by laser capture dissection, and subsequently mRNAs encoding catecholamine receptors identified by qPCR. Layer 6 (L6) PCs that project to the thalamic mediodorsal nucleus (MD) express high levels of D1 but very low levels of D2 mRNA. L5 PFC neurons that innervate MD express the D1 but not D2 receptor. In contrast, L5 PFC neurons that

innervate the ventral tegmental area (VTA) express high levels of D2 but not D1 mRNA. Layer 5 PFC that innervate the VTA exhibit very little alpha2a receptor mRNA but express very levels of the alpha2c receptor transcript. The pattern of various catecholamine receptors expressed by laminar- and target-specific PFC PCs may offer unique insights into the mechanisms underlying dopamine denervation-induced dendritic remodeling of PFC projection neurons.

Disclosures: H. Wang: None. **M.J.M. Murphy:** None. **M.S. Simmons:** None. **J.H. Meador-Woodruff:** None. **A.Y. Deutch:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

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Title: Pre-training lesion to dorsal Hippocampus changes connectivity among amygdala subnuclei after contextual fear conditioning

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[1]Psicobiologia, Univ. Federal De Sao Paulo, Sao Paulo, Brazil; [2]Ctr. de Matemática, Computação e Cognição, Univ. Federal do ABC, Santo André, Brazil

Abstract: In Contextual Fear Conditioning (CFC), dorsal hippocampus (dHPC) is thought to send context information to amygdala (AMY) to serve as CS in the CS-US association. However, pre-training lesion to dHPC does not impair CFC while it does in other spatial tasks. Hypothesis have been raised on whether context representations remain the same in such case. Because AMY receives anatomical inputs of different modalities (i.e. unimodal and multimodal stimuli) in different nuclei, if dHPC lesioned animals still learn context as normal animals, differences in nuclei activity and/or network relations should not occur after CFC. Here, we

investigated how pre-training bilateral dHPC eletrolitic lesion affects pCREB expression in the dorsolateral (LADL), ventrolateral (LAVL) and ventromedial (LAVM) parts of lateral nucleus, and anterior (BLA), posterior (BLP) and ventral (BLV) parts of basal nucleus of amygdala, as well as network relations among these subnuclei. In experiment 1, to ensure the dHPC lesions were effective, dHPC and SHAM rats were submitted to the Water Maze (WM) and, 10 days later, to CFC. The dHPC group showed impaired learning during training and impaired memory retrieval on the test day, while did not show any impairment on CFC, compared to SHAM group. In experiment 2, dHPC and SHAM group were submitted to the CFC with an additional SHAM group submitted to an immediate shock (S) protocol. Half the rats were perfused with paraformaldehyde after 3h. Their brains were processed for immunohitochemical (IHC) analysis (with additional naive rats) and pCREB expression was evaluated in LADL, LAVL, LAVM, BLA, BLP, BLV. The other half was tested for fear memory 48h later. DHPC did not show any contextual fear deficit, neither they did differ from SHAM in pCREB expression in any amygdala subnuclei, although both differed from S group in LADL and SHAM differed from S in BLA. The lack of difference between dHPC and S in BLA, BLP or BLV, that are known to receive multimodal inputs, suggests that context representation may not be associated to US as it is in SHAM group. A correlation matrix for all subnuclei was used in a network analysis in which we compared the weighted degree and eigenvector centrality of measured regions between dHPC and SHAM groups. A bootstrap procedure was performed to obtain a random distribution and p-value for this comparison and assess its significance. SHAM group showed a higher eigenvector centrality and weighted degree in BLV than dHPC. This difference in nuclei centrality suggests that what is learned (associated to US) in terms of context is likely to be different in dHPC lesioned animals.

Disclosures: **C.A. Coelho:** None. **J.R. Sato:** None. **M.M. Oliveira:** None. **J.C.K. Soares:** None. **T.L. Ferreira:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 754.02/UU64

Topic: F.02. Animal Cognition and Behavior

Title: Epigenetic mechanisms mediating contextual fear conditioning and generalization in the hippocampus

Authors: *J. C. HEBERT[1], R. K. YUAN[2], I. A. MUZZIO[2];
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Abstract: The use of epigenetic modifiers as cognitive enhancers has received much attention in the past decade. Recently, it has been shown that inhibition of histone deacetylase (HDAC) in the dorsal hippocampus enhances contextual fear conditioning in mice. However, the differential effects of HDAC inhibition in the dorsal and ventral hippocampus have not yet been investigated. Since these regions are thought to play distinct roles in the encoding of context, HDAC inhibition in these specific regions may have differential effects on fear conditioning and generalization. We hypothesize that HDAC inhibition in the dorsal hippocampus will enhance learning as previously shown, while HDAC inhibition in the ventral hippocampus will lead to increased fear generalization. In this study, mice received bilateral infusions of MS-275, a class I HDAC inhibitor, in either the dorsal or ventral hippocampus immediately after context pre-exposure or after the associative phase of predator-odor contextual fear conditioning. Subsequent retrieval tests were performed 24 hours later in the conditioning context and a novel context. We found that dorsal infusions of MS-275 immediately after context pre-exposure enhanced fear learning in the conditioning context and produced a trend toward generalization in the novel context, while infusions after the associative phase had no effect. These results confirm that the main role of the dorsal hippocampus in contextual fear is to form a representation of context and that this process is enhanced by epigenetic mechanisms. We are currently extending our findings to the ventral hippocampus. The results may elucidate distinct functions of the dorsal and ventral hippocampus in contextual fear conditioning as well as the mechanisms underlying fear generalization.

Disclosures: J.C. Hebert: None. **R.K. Yuan:** None. **I.A. Muzzio:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 754.03/UU65

Topic: F.02. Animal Cognition and Behavior

Support: R01MH065961

Title: Subicular and CA1 neurons projecting to the medial prefrontal cortex and basal amygdala exhibit context-dependent Fos expression after renewal of extinguished fear

Authors: *J. JIN[1], S. MAREN[1,2];
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Abstract: The ventral subiculum (vSUB) and hippocampus CA1 (vCA1) are believed to play a critical role in the relapse or renewal of extinguished fear memories. Projections from these areas target both the medial prefrontal cortex and amygdala, but the relative contribution of these projections is not clear. Here we used Fos immunohistochemistry along with retrograde tracers to characterize retrieval-related activity in vSUB and vCA1 neurons projecting to either the prelimbic cortex (PL) or basal amygdala (BA) or both. Prior to fear conditioning, rats were injected with distinct retrograde tracers into PL and BA to retrogradely label vSUB and vCA1 projection neurons. After fear conditioning and extinction, rats were tested for their fear (indexed by freezing behavior) to an auditory conditioned stimulus (CS) either inside the extinction context or in a different context; one group of animals underwent conditioning and extinction but was not tested. All rats were sacrificed 90 minutes after the retrieval test. Presenting the CS outside the extinction context renewed conditioned fear and was associated with c-Fos expression in the vSUB and vCA1. Interestingly, Fos was induced in the vCA1, but not vSUB, when the retrieval test was conducted in the extinction context. However, within the vSUB and vCA1, neurons projecting to PL, BA, or both exhibited context-dependent Fos activity, with lower levels of Fos expression after testing in the extinction context relative to testing outside that context. Interestingly, dual-projecting neurons in vSUB and vCA1 exhibited greater levels of renewal-related Fos expression than neurons projecting to either PL or BA alone. These data suggest that the contextual retrieveal of fear memories is differentially regulated by the vSUB and vCA1. Nonetheless, PL and BA projecting neurons in these areas appear to make similar contributions to the context-dependent expression of extinguished fear.

Disclosures: J. Jin: A. Employment/Salary (full or part-time):; Texas A&M University. **S. Maren:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

Location: Halls A-C

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Program#/Poster#: 754.04/UU66

Topic: F.02. Animal Cognition and Behavior

Support: Planned Collaborative Research Center 1080, Germany.

Title: Gadd45a plays a crucial role in the consolidation of hippocampal-dependent memories

Authors: A. APARISI REY[1], M. S. GIERL[3], S. SHAROPOV[2], E. KARAULANOV[3], S. GUGGENHUBER[1], F. REMMERS[1], W. H. GRUHN[3], *W. KILB[4], A. CONRAD[1], H. J. LUHMANN[2], C. NIEHRS[3], B. LUTZ[1];
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Abstract: The Growth Arrest and DNA Damage-inducible 45 (Gadd45) proteins have been implicated in a broad variety of cellular functions including active DNA demethylation and regulation of Mitogen-Activated Protein Kinases (MAPKs) among others. Recently, Gadd45 proteins have been shown to be involved in neuronal plasticity and epigenetic gene activation, processes that can underlie memory formation. In our study, we initially performed a comprehensive evaluation of the behavior of Gadd45a-deficient mice (Gadd45a-KO), finding a specific deleterious effect on consolidation of hippocampal-dependent memories in 2 different behavioral paradigms. Moreover, activation of Extracellular-signal Regulated Kinases (ERK) within the first hours after the memory acquisition, constituting a hallmark of synaptic plasticity, was significantly delayed in Gadd45a-KO mice. Additionally, electrophysiological recordings of hippocampal slices of Gadd45a-KO mice showed a reduced long-term potentiation (LTP) as compared to their wild-type littermates. Remarkably, the overexpression of Gadd45a in glutamatergic neurons of the hippocampus, mediated by intracranial injections of an adeno-associated virus containing the *Gadd45a* gene, led to a phenotype characterized by an enhanced memory formation, higher levels of MAPK activation and a minor, but significant increase in late LTP. Genome-wide gene expression analysis of Gadd45a-KO mice versus controls is ongoing and is aimed at revealing the molecular mechanisms underlying the memory phenotype observed in these mutant mice. Our study contributes to the understanding of the role of Gadd45 proteins in brain functions, proposing Gadd45a as a fundamental regulator of synaptic plasticity and memory formation.

Disclosures: A. Aparisi Rey: None. **M.S. Gierl:** None. **S. Sharopov:** None. **E. Karaulanov:** None. **S. Guggenhuber:** None. **F. Remmers:** None. **W.H. Gruhn:** None. **W. Kilb:** None. **A. Conrad:** None. **H.J. Luhmann:** None. **C. Niehrs:** None. **B. Lutz:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

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Program#/Poster#: 754.05/UU67

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Narsad Young Investigator Award to N.S.

CIHR fellowship to N.S.

FRSQ fellowship to N.S.

Title: Fibroblast growth factor 2 modulates anxiety behavior and the hippocampal-hypothalamic pituitary axis

Authors: *N. SALMASO[1], M. EL SAYED[2], Q. REN[1], M. E. MARAGNOLI[2], R. M. SAPOLSKY[3], R. DUMAN[2], F. M. VACCARINO[1];
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Abstract: Fibroblast growth factor 2 (fgf2) levels have been negatively correlated with anxiety/depressive behavior both in rodents and in patients with mood disorders. We demonstrate that the absence of a functional fgf2 gene contributes to the etiology of increased anxiety, decreased hippocampal glucocorticoid receptor (GR) expression, and increased hypothalamic-pituitary-adrenal axis (HPA) activity. Furthermore, this behavioral phenotype is specific to anxiety behavior, as no changes were observed in depressive or locomotor behavior. Fgf2 administration in adulthood was sufficient to rescue this phenotype, and was strongly related to regulation of HPA perturbations. Blockade of GR in adult mice treated with FGF2 blocked the therapeutic effects of FGF2 on anxiety behavior, suggesting that fgf2 is acting through GR to exert its effects on anxiety behavior. Because the transcription factor egr-1 is an important modulator of GR transcription, and because egr-1 is downstream of fgf2 signaling, we examined whether changes in hippocampal egr-1 expression and found decreased levels in KO mice that were reestablished with FGF2 treatment. To verify if these changes in egr-1 were related to changes in GR expression, we performed chromatin immunoprecipitation of the egr-1 antibody in wildtype and knockout mice, with and without fgf2 rescue. Together, these data suggest that FGF2 levels are critically related to anxiety behavior and HPA activity, likely through modulation of hippocampal glucocorticoid receptor expression.

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Poster

754. Fear and Aversive Memories: Hippocampus

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01-DA027305

Title: Long-range GABAergic neurons participate in reciprocal interconnections between the rat basolateral amygdala and the parahippocampal region, but not the prefrontal cortex

Authors: *A. J. MCDONALD, V. ZARIC;
Univ. South Carolina Sch. Med., COLUMBIA, SC

Abstract: Connections of the basolateral amygdalar nuclear complex (BLC) with the hippocampal region and prefrontal cortex (PFC) are critical for the formation and extinction of fear memories. Although it is well established that glutamatergic pyramidal cells are the main cell type involved in these connections, several studies have found evidence that nonpyramidal neurons (NPNs), including GABAergic neurons, may also be involved. In the present study Fluorogold (FG) tract tracing was combined with fluorescence immunohistochemistry for NPN markers to investigate this question. Markers included GABA, GAD, somatostatin (SOM), NPY, parvalbumin (PV), vasoactive intestinal peptide (VIP), and the m2 muscarinic receptor. Injections of FG into the BLC produced widespread retrograde labeling in the cerebral hemispheres and diencephalon. FG/SOM/NPY triple-labeling revealed a small number of FG+/SOM+/NPY+ neurons and FG+/SOM+/NPY- neurons in the lateral entorhinal area (LERA) amygdalopiriform transition area, and piriform cortex, but not in the PFC or diencephalon. In addition, clusters of FG+/SOM+/NPY+ neurons were observed in the amygdalostriatal transition area and surrounding the rostral intercalated nuclei. The latter neurons appear to correspond to the “SPIN” neurons described in previous studies. About half of the SOM+ neurons in the LERA labeled by FG were GABA+. FG+/PV+ neurons were only seen in the basal forebrain and no FG+/VIP+ neurons were observed in any brain region. To determine if NPNs in the BLC project to cortical areas, FG injections were made into the LERA or PFC. FG/SOM/NPY triple-labeling revealed a small number of FG+/SOM+/NPY+ neurons and FG+/SOM+/NPY- neurons in the BLC with FG injections into the LERA, but not with injections into the PFC. About half of the SOM+ neurons in the BLC labeled by FG injections into the LERA were m2R+, 62% were GAD+, but none were PV+ or VIP+. These data indicate that NPNs, including GABAergic neurons, participate in reciprocal interconnections between the BLC and the parahippocampal region, including the LERA, but not with the PFC. These long-range NPNs include subpopulations of SOM+ and NPY+ neurons, but not PV+ or VIP+ neurons. Determination of the targets of these neurons will be important for understanding their functional role. Since long-range NPNs involved in corticocortical connections are critical for synchronous oscillations that allow temporal coordination between distant brain regions, the long-range NPNs identified in

this study may play a role in the synchronous oscillations of the BLC and the hippocampal region involved in retrieval of fear memories.

Disclosures: A.J. McDonald: None. **V. Zaric:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Grants-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant no. 26780419)

Research Grant for Public Health Science

New ・ frontier ・ project

Title: Learning-dependent synaptic diversity in hippocampal CA1 neurons: Encoding of context but not retrieval induces rapid plasticity at excitatory and inhibitory synapses

Authors: *Y. SAKIMOTO, D. MITSUSHIMA;
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Abstract: Recently, we reported that contextual learning with retrieval test strengthens both excitatory and inhibitory CA1 synapses, showing wide diversity of post-synaptic currents in each CA1 neuron (Mitsushima et al, Nature Communications 2013). To determine whether the encoding of context or retrieval induces the synaptic diversity and elucidate the timing of plasticity, we analyzed the postsynaptic miniature currents using patch clamp method under the presence of TTX (0.5μM). Rats were trained inhibitory avoidance (IA) task, and acute brain slices were prepared in untrained, IA-trained, or IA-trained rats after retrieval test. By changing the membrane potential, we recorded miniature EPSCs (at -60 mV) and miniature IPSCs (at 0 mV) sequentially from the same CA1 neuron. Although untrained rats showed relatively small mEPSC and mIPSC amplitudes with low diversity of post-synaptic currents, both IA-trained and IA-trained with retrieval rats consistently showed higher mEPSC and mIPSC amplitudes with wide diversity. The levels in IA-trained rats are not significantly different from those in IA-trained rats with retrieval test, suggesting encoding of context rather than retrieval induces the synaptic plasticity and diversity. Moreover, the synaptic plasticity and diversity rapidly induced

within 10 min after the IA training. Despite the synaptic diversity, mean excitatory and inhibitory inputs consistently balanced in all experimental groups. Further, bath application of CNQX (an AMPA receptor antagonist, 10 μM) or bicuculline methiodide (a GABAA receptor antagonist, 10 μM) consistently blocked the mEPSC or mIPSC events, respectively. These results suggest that the encoding rather than retrieval of context drives rapid synaptic delivery of AMPA receptors and GABAA receptors at CA1 synapses within 10 min after the contextual training.

Disclosures: Y. Sakimoto: None. **D. Mitsushima:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

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Support: CREST, JST

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Title: Artificial association of information residing in hippocampus and amygdala

Authors: *N. OHKAWA[1,3], Y. SAITOH[1,3], A. SUZUKI[1,3], S. TSUJIMURA[1,3], E. MURAYAMA[1,3], H. NISHIZONO[2], M. MATSUO[2], Y. TAKAHASHI[4], M. NAGASE[4], Y. K. SUGIMURA[4], A. M. WATABE[4,5], F. KATO[4,5], K. INOKUCHI[1,3];
[1]Dept. of Biochem, Grad. Sch. of Med. and Pharm., [2]Div. of Animal Exptl. Laboratory, Life Sci. Res. Ctr., Univ. of Toyama, Toyama, Japan; [3]CREST, JST, Toyama, Japan; [4]Dept. of Neurosci., Jikei Univ. Sch. of Med., Tokyo, Japan; [5]Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan

Abstract: Memory is assumed to be stored in the brain as a cellular ensemble consisting of a set of neurons that is activated during learning. Although optical stimulation of a cellular ensemble is known to trigger the retrieval of the corresponding memory, it is unclear how the association of distinct information occurs at the cell ensemble level. Here, we show in mice that activation of a cell ensemble corresponding to two distinct memory events generates an artificial association between initially non-related events. In the context pre-exposure and immediate shock (IS) paradigm, mice failed to associate the shock with the pre-exposed context when the IS was delivered to their foot in a different context. Cells activated during the context pre-exposure and the IS in hippocampal CA1 and the basolateral amygdala (BLA) were targeted with channelrhodopsin-2, a light-activated cation channel. These cells were later simultaneously activated by optical stimulation in the mice's home cage. The next day, these mice exhibited freezing behaviour, an indicator of a fear response, in the pre-exposed context that was not originally associated with the shock. Thus, the artificial activation of distinct cell ensembles, without any sensory input, is capable of generating an artificially associated memory. Furthermore, our finding suggests that the association of distinct units of information is achieved through the synchronous activity of distinct cell ensembles. This mechanism may underlie memory update by incorporating novel information into pre-existing networks to form qualitatively new memories.

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Poster

754. Fear and Aversive Memories: Hippocampus

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Support: NHMRC (Australia)

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Victorian Government Operational Infrastructure Support Programme

Title: Relaxin-3/RXFP-3 signalling in control of innate and learned affective behaviours - effect of acute and chronic RXFP3 modulation and role of ventral hippocampus

Authors: *V. RYTOVA, D. HAWKES, D. E. GANELLA, S. H. ONG, F. SHABANPOOR, M. A. HOSSAIN, J. D. WADE, R. A. D. BATHGATE, S. MA, A. L. GUNDLACH;
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Abstract: Relaxin-3, a neuropeptide abundantly expressed by GABAergic neurons in the nucleus incertus (NI), preferentially binds/activates a G_i-protein-coupled receptor, RXFP3. NI relaxin-3 neurons express CRF receptors, are stress-responsive, and constitute a conserved ascending network in rodent and primate brain, enriched in limbic areas involved in fear and anxiety-related behaviours, such as extended amygdala, BNST, ventral hippocampus (vHPC), and prefrontal cortex. Our research has shown relaxin-3/RXFP3 signalling modulates memory processing, wakefulness and arousal, stress, alcohol seeking, depressive- and anxiety-like behaviours. Our current goal is to characterize how relaxin-3/RXFP3 signalling in various limbic regions modulates 'affective' behaviour including innate anxiety and learned fear in the rat. We first examined effects of a specific RXFP3 agonist, RXFP3-A2, in an auditory fear conditioning paradigm. Central RXFP3 activation by intracerebroventricular (icv) injection of RXFP3-A2 (5-15 μg) in adult Sprague-Dawley rats (n=5-7/group) resulted in dose-related impairment of fear extinction associated with altered neural activation (Fos-staining) patterns in limbic regions. In contrast, icv RXFP3-A2 injection reduced innate anxiety in the elevated plus maze (EPM) and light/dark box (LDB) tests, and depressive-like indices (immobility) in the forced swim test [Ryan PJ *et al*., 2013a]. These and other data [Ryan PJ *et al.* 2013b] suggest complex 'topographic' effects of RXFP3 signalling on fear, mood and anxiety, related to precise site(s) and timing of endogenous and exogenous peptide action. In further studies, we injected AAV1/2 constructs, which drive local secretion of RXFP3 agonist (R3/I5) or antagonist (R3(1-22)R) peptides, into the vHPC (n=6/group), and examined the effects of RXFP3 activation/inhibition on performance in the LDB, EPM and large open-field (LOF). Chronic RXFP3 activation in vHPC decreased the time spent (91% decrease, p=0.011) and distance travelled (88% decrease, p=0.008) in the light zone of LDB, and produced trends for increased anxiety in EPM and LOF *c.f* control vector. We are now assessing the impact of chronic activation/inactivation of vHPC RXFP3 on social interaction, spatial learning, and fear conditioning and will assess key neurochemical indices in the absence or presence of the viral constructs, post-mortem. This research will better characterise the impact of this widespread neuropeptide/receptor system and associated neural circuits on complex affective behaviours, with implications for understanding anxiety/mood disorders and potential therapeutic targets.

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Poster

754. Fear and Aversive Memories: Hippocampus

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: W81XWH-08-1-0661

Title: Effects of single-prolonged stress on adult hippocampal neurogenesis

Authors: *E. RODRIGUEZ[1], I. LIBERZON[2];
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Abstract: Functionally, posttraumatic stress disorder (PTSD) has been linked to impairments in fear extinction retention and contextual processing, dependent on intact prefrontal cortex-hippocampus circuitry. Hippocampus is critical for processing of contextual information, and fear extinction depends on the context. Interestingly, PTSD patients have been reported to have hippocampus functional and volumetric abnormalities. At the cellular level, adult hippocampal neurogenesis plays a role in key hippocampal functions. Furthermore, neurogenesis is sensitive to stress suggesting a link between traumatic stress, hippocampal neurogenesis and deficits in contextual processing. Here we investigate if our PTSD model- single prolonged stress- affects adult hippocampal neurogenesis and how it relates to context-dependent fear processing. Rats were either undisturbed or exposed to SPS (two-hour restraint, 20min forced-swim and ether exposure). Experiment-1: rats' brains were processed for the immature neuronal marker-doublecortin (DCX). Experiment-2: controls and SPS were fear-conditioned, extinguished, then tested for extinction retention. After, all were intraperitoneal injected 50mg/kg of BrdU for seven days. Brains were then processed for BrdU immunohistochemistry and their freezing behavior was analyzed. SPS decreased dentate gyrus DCX+ cells compared to controls ($t= 2.7$, $P= 0.017$). There was a negative correlation between the number of BrdU+ cells and the lowest percentage of time spent freezing during extinction ($r(6)=-0.71$, $P=0.049$) in SPS rats, but not controls. These results suggest that SPS exposure may lead to a decrease in hippocampal neurogenesis and that the degree to which it is reduced may play a direct role in fear extinction learning.

Disclosures: E. Rodriguez: None. **I. Liberzon:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 754.11/UU73

Topic: F.02. Animal Cognition and Behavior

Title: Learning-dependent synaptic plasticity at CA1 synapses: Laterality and a possible location of contextual memory in the hippocampus

Authors: *J. MIZUNO[1], Y. SAKIMOTO[2], H. KIDA[2], Y. ONO[3], Y. KAMIYA[4], D. MITSUSHIMA[2,1];
[1]Physiol., Kanagawa Dent. Univ., Yokosuka / Kanagawa, Japan; [2]Dept. of Systems Neurosci., Yamaguchi University, Grad. Sch. of Med., Ube, Japan; [3]Dept. of Electronics and Bioinformatics, Meiji Univ. Sch. of Sci. and Technol., Kawasaki, Japan; [4]Dept. of Anesthesiol., Niigata Univ. Grad. Sch. of Med., Niigata, Japan

Abstract: We previously showed the synaptic delivery is required for contextual learning, since bilateral expression of AMPA receptor delivery blocker successfully impaired the contextual learning (*PNAS* 108: 12503-508, 2011). However, the location of contextual memory is still unclear, since there are functional dissociations between the dorsal and ventral hippocampus, and the left-right asymmetry of hippocampal circuitry. In experiment I, to determine a possible location of learning-dependent synaptic plasticity, we made acute brain slices in four different CA1 areas in untrained or IA-trained rats. Then, we stimulated CA3-CA1 synapses to measure the ratio of AMPA receptor to NMDA-type receptor responses (A/N) using voltage-clamp methods. In the dorsal hippocampus, IA-trained rats showed significantly higher A/N ratio than untrained rats. Moreover, the change in the left CA1 was not significantly different from the change in the right CA1. Conversely, in the ventral hippocampus, A/N ratio showed no significant learning-dependent change in both sides. Using non-stationary noise-analysis techniques, we further analyzed the estimated number of AMPA receptors. The estimated number of AMPA receptors in IA-trained rats was significantly larger than that in untrained rats in the dorsal hippocampus. In experiment II, to further analyze the learning-dependent synaptic plasticity, we recorded miniature responses under the presence of 0.5 μM TTX in both sides of the dorsal hippocampus. By changing the membrane potential, we recorded miniature EPSCs (at -60 mV) and miniature IPSCs (at 0 mV) sequentially from the same CA1 neuron, as we reported previously (*Nat Commun*, 4:2760 doi: 10.1038/ ncomms3760, 2013). Although untrained rats showed small mEPSC and mIPSC amplitudes, IA-trained rats consistently showed higher mEPSC and mIPSC amplitudes in both sides. Moreover, the change in the left CA1 was not

significantly different from the change in the right CA1. Bath application of CNQX (an AMPA receptor antagonist, 10 μM) or bicuculline methiodide (a $GABA_A$ receptor antagonist, 10 μM) consistently blocked the mEPSC or mIPSC events, respectively. In experiment III, to examine learning-dependent changes in both sides of dorsal CA1 neurons, intrinsic neural property was further analyzed using current-clamp methods. Neither IA-training nor side affected series resistance, input resistance, and resting membrane potential in CA1 neurons. These results suggest that learning-dependent synaptic delivery of AMPA receptors at CA1 synapses occur in the dorsal hippocampus bilaterally, regardless of the left or right hippocampus, but not in the ventral hippocampus.

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Poster

754. Fear and Aversive Memories: Hippocampus

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 5R00MH083943

NIH Grant R01MH102595

Title: Adult hippocampal neurogenesis and trace fear conditioning: A role in parceling associative fear among ambiguous competing cues?

Authors: *D.-O. SEO[1], M. NGUYEN[1], F. SHUE[1], M. A. CARILLO[2], R. HEN[2], M. R. DREW[1]; [1]The Univ. of Texas At Austin, Austin, TX; [2]Columbia Univ., New York City, NY

Abstract: Trace conditioning rescues newborn hippocampal neurons from death (e.g., Gould et al., 1999; Shors et al., 2001), but are newborn neurons necessary for trace conditioning? Studies suggest that arresting adult neurogenesis impairs trace conditioning, but these studies used methods that arrest cell proliferation throughout the body, not just neurogenesis in the hippocampus. We revisited the role of adult neurogenesis in trace conditioning using methods that may provide a more specific arrest of adult hippocampal neurogenesis. One method is targeted, low-dose x-irradiation. The second is a novel transgenic mouse, the DCX-TK mouse, in which an inducible suicide gene is expressed specifically in newborn neurons and their lineage-

restricted progenitors. Irradiated and sham-irradiated mice were conditioned 6-8 weeks following irradiation using delay or trace fear conditioning procedures. Irradiated and control mice displayed similar levels of tone fear in both delay and trace procedures. However, an unexpected difference in context-elicited fear emerged. In the trace but not the delay procedure irradiated mice exhibited significantly more context fear than controls. To confirm this unexpected phenotype, DCX-TK and WT mice were trace conditioned after the inducible arrest of neurogenesis. Similar to irradiated mice, DCX-TK mice displayed normal fear of the trace CS but increased context fear compared to WT controls. The enhanced contextual fear in DCX-TK was again specific to hippocampus-dependent trace conditioning. When DCX-TK and WT mice were given delay fear conditioning, or a hippocampus-independent version of trace conditioning, DCX-TK and WT mice exhibited similar levels of context fear. Follow-up experiments suggest that the enhanced context fear in mice lacking neurogenesis stems from an impairment in parceling associative fear among competing cues. This impairment may arise through two mechanisms. First, in procedures such as trace conditioning in which the associative status of the context and the tone CS is ambiguous, mice lacking neurogenesis attribute associative strength to all potential predictive stimuli, whereas in control mice the strongest predictive stimulus overshadows weaker predictive stimuli. A second possibility is that arrest of neurogenesis impairs conditioning to the trace CS, but this impairment is masked by nonspecific fear sensitization (e.g., Kamprath & Wotjak, 2004). As previously reported, and confirmed in our studies, fear conditioning in mice can produce considerable non-associative fear of the conditioned stimulus. Experiments in progress will distinguish among these potential explanations.

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Poster

754. Fear and Aversive Memories: Hippocampus

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 754.13/UU75

Topic: F.02. Animal Cognition and Behavior

Support: RIKEN

HHMI

Title: Bidirectional reversal of the valence associated with the hippocampal memory engram

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Abstract: The valence of memories is malleable and this reconstructive property of memory is used clinically to treat maladaptive behaviours. However, the neuronal mechanisms and brain circuits that enable the switching of the valence of memories remain largely unknown. Here, we investigated the mechanisms underlying the reversal of memory valence by applying optogenetics to mice. A population of neurons active during a fearful or rewarding experience was labelled with Channelrhodopsin-2 (ChR2) in the dorsal dentate gyrus (DG) of the hippocampus, or in the basolateral complex of the amygdala (BLA) in mice. In both the DG- and BLA- labelled mice, subsequent light-induced reactivation of neurons labelled during a fear experience elicited an avoidance response, whereas similar reactivation of neurons that were labelled during a reward experience elicited an appetitive response. Next, to reverse the valence of the memory engram, we optogenetically activated fear- or reward-labelled neurons during an experience of the opposite valence. Subsequent optogenetic stimulation of the DG, but not the BLA, elicited a behavioural response in accordance with the experience during the optogenetic reactivation and opposite to the activation of the original engram. This switch in valence was also evidenced at the cellular level by a change in functional connectivity between the DG-engram and the BLA-engram. Additionally, the reversal of the valence associated with the DG engram was sufficient to alter the original memory. Thus, we have found that in the hippocampal DG, but in the BLA, the neurons active during the encoding of an associative memory show the plasticity sufficient to rewrite the valence associated with their firing. Our present work provides new insight into the functional neural circuit underlying the malleability of emotional memory.

Disclosures: R.L. Redondo: None. **J. Kim:** None. **A.L. Arons:** None. **S. Ramirez:** None. **X. Liu:** None. **S. Tonegawa:** None.

Poster

754. Fear and Aversive Memories: Hippocampus

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Program#/Poster#: 754.14/UU76

Topic: F.02. Animal Cognition and Behavior

Title: Metformin injections into the hippocampus facilitate the extinction of cocaine associated drug memory

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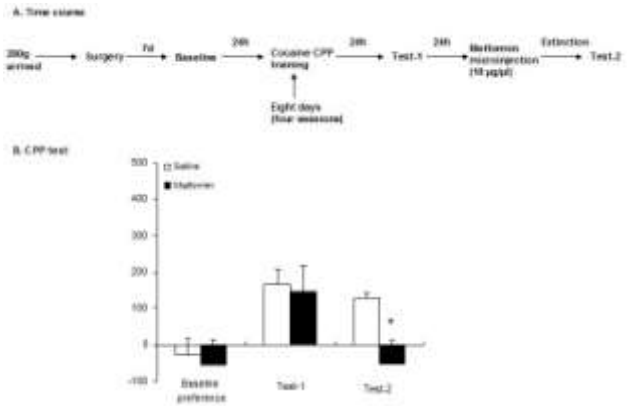
Abstract: Drug addiction is becoming an economic and social problem which cannot be ignored. After drug taking, a persistent pathological rewarding memory will be formed with drug associated cues and contexts, which is the crucial factor that leads to drug relapsing in drug addicts. The pharmacological methods are used to promote the extinction of the pathologocal rewarding memory, which are the effective methods to prevent or treat drug relapsing. Introperitoneal injection of metformin can facilitate neurogenesis in the hippocampus of mice, while the neurogenesis in the hippocampus plays an important role in learning and memory, such as ablation of the neurogenesis in the hippocampus in mice impaired extinction of contextual fear memory. It is unclear whether metformin play an crucial role in the extinction of pathological rewarding memory through regulating neurogenesis in the hippocampus. Microinjection of metformin will be used in our experiments to facilitate the neurogenesis in the hippocampus in rats during the extinction of cocaine and heroin associated rewarding memory and we want to determine the role of metformin in the extinction of pathological rewarding memory. Microinjections of metformin into hippocampus during cocaine CPP extinction facilitate the extinction of cocaine CPP. Our experiments demonstrate that metformin regulated the extinction of cocaine associated drug memory through affecting the neurogenesis in the hippocampus.



Disclosures: **Z. Ding:** None. **S. Sun:** None. **L. Lu:** None.

Poster

755. Reward: Dopamine II

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Support: NSERC CGS Doctoral Scholarship

Concordia University Graduate Fellowship

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Title: Optogenetic excitation of midbrain dopamine neurons and an afferent pathway: Implications of electrochemical and behavioral sequallae for the descending-path hypothesis

Authors: *M.-P. COSSETTE[1], I. TRUJILLO-PISANTY[1], I. WITTEN[2], K. DEISSEROTH[3], P. SHIZGAL[1];
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Abstract: Midbrain dopamine (DA) neurons have long been implicated in the rewarding effects of natural goal objects, abused drugs and electrical brain stimulation. Initially, these DA neurons were thought to be excited directly in intra-cranial self-stimulation (ICSS) experiments. However, the fine unmyelinated axons of DA neurons have high thresholds to extracellular stimulation, and their excitability and conduction properties differ from the inferred characteristics of the directly stimulated neurons subserving ICSS of the medial forebrain bundle (MFB). Behavioral measurements of recovery from refractoriness, collision, and anodal hyperpolarization block implicate rostrocaudally projecting, myelinated LH-VTA fibers in the rewarding effect produced by electrical stimulation of the MFB (the "descending-path hypothesis"). We delivered 1s trains of 5 ms, 473 nm optical pulses to the lateral hypothalamus (LH) or ventral tegmental area (VTA) of urethane-anesthetized rats expressing channelrhodopsin2 (ChR2) in LH neurons under control of the CaMKIIa promoter. Fast-scan cyclic voltammetry (FSCV) revealed that DA transients were evoked in the nucleus accumbens (NAc) terminal field by optical stimulation of either the LH or VTA. This is consistent with prior results in mice and with the descending-path hypothesis. Transposition to the MFB of Moisan and Rompré's model of the circuitry subserving ICSS of the posterior mesencephalon assigns to the midbrain DA neurons a role either in the spatiotemporal integration of inputs from the

directly stimulated ICSS substrate or in a downstream stage of processing. If so, at least two stages of integration contribute to the rewarding effect: one comprising, or upstream from, the midbrain DA neurons and another at, or beyond, the forebrain terminal fields of these neurons. We obtained both electrochemical and behavioral data pertinent to the downstream integrator. In urethane-anesthetized, TH::Cre rats that had received prior VTA injections of an AAV5 virus containing an EF1a-DIO-hChR2(H134R)-EYFP transcript, we delivered 1s trains of 5 ms, 473 nm, optical pulses to midbrain DA neurons expressing ChR2 and recorded the resulting DA transients in the NAc by means of FSCV. The peak amplitude of the DA transients is a joint function of optical power and pulse frequency, as if determined by the aggregate rate of optically induced firing. In a parallel behavioral experiment in TH::Cre rats, we found that optical power and pulse frequency trade off in supporting lever pressing for optical stimulation of VTA DA neurons expressing ChR2. Thus, the aggregate optically induced spike rate also appears to determine the rewarding effect.

Disclosures: M. Cossette: None. **I. Trujillo-Pisanty:** None. **I. Witten:** None. **K. Deisseroth:** None. **P. Shizgal:** None.

Poster

755. Reward: Dopamine II

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CONACYT scholarship to ITP

PBEE-MELS scholarship to ITP

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Title: Three dimensional modeling of optical self-stimulation of midbrain dopamine neurons in TH::Cre rats

Authors: *I. TRUJILLO-PISANTY[1], D. PALACIOS[2], M.-P. COSSETTE[2], K. CONOVER[2], I. WITTEN[3], K. DEISSEROTH[4], P. SHIZGAL[2];

[1]Psychology (CSBN), [2]Concordia Univ., Montreal, QC, Canada; [3]Princeton Univ., Princeton, NJ; [4]Stanford Univ., Stanford, CA

Abstract: Optogenetic methods play a vital role in linking neural circuitry to behavioral functions by providing an unprecedented combination of specificity and temporal resolution. Neural activity is induced or silenced on appropriate timescales, in targeted cellular populations, thereby demonstrating the sufficiency and necessity of that activity for the observed behavioral output. The significance of such findings depends on the adequacy and power of the behavioral methods employed and on the computational framework that links behavioral observations to neural signals. We measured the allocation of reward-seeking behavior as a function of the strength and cost of an optogenetically generated reward: optical stimulation of midbrain dopamine (DA) neurons. The results shed light on the integration of DA output and how the resulting reward signal is combined with the subjective opportunity cost of reward-seeking behavior. A Cre-dependent virus (AAV5 Ef1a-DIO-ChR2-eYFP) was injected bilaterally into the ventral tegmental area (VTA) of TH::Cre rats. Optical fibers were positioned to illuminate the injection sites. At least 5 weeks post-surgery, the rats were trained to hold down a lever for optical stimulation (1 s trains of 473 nm, 5 ms pulses). When the cumulative hold time reached an experimenter-defined criterion (the reward "price"), the optical reward was delivered. Reward strength was manipulated by varying the optical pulse frequency. The rats worked vigorously for strong and cheap rewards but systematically allocated time to competing activities as reward strength decreased and/or price increased. These results are well described by a computational model derived from earlier studies of electrical self-stimulation of VTA input pathways that activate DA neurons transsynaptically. In this model, subjective reward intensity grows logistically with the induced spike frequency and undergoes scalar combination with subjective reward cost. That the model applies both in the case of direct and indirect activation of VTA DA neurons suggests that spatiotemporal integration of reward-related signals occurs both upstream and downstream from the DA neurons and that cost information is incorporated beyond the output of the downstream integrator. The model can help determine the locus of action of treatments that alter performance for optical reward while accounting for differences and similarities between optical and electrical self-stimulation.

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Poster

755. Reward: Dopamine II

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Support: NSFC 31271169

NSFC 91132702

Title: Parallel excitatory and inhibitory pathways of basal ganglia and lateral habenula underlie the learned responses under stochastic schedule of reward

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Abstract: Efficiently perceiving the uncertainty is critical to make a better survival in a world full of uncertainty. To investigate the underlying neural mechanism, recordings from well trained animals applying the stochastic schedule of reward have been extensively carried out. These experiments demonstrate that dopaminergic (DA) neurons and lateral habenula (LHb) neurons play important roles in the perception of uncertainty. For the reward conditional stimulus (reward CS), the DA (LHb) neurons exhibit a phasic peak (dip) activity upon CS. The DA (LHb) neurons show a base line in response to reward outcome but a phasic dip (peak) in response to the nonreward outcome. For the nonreward CS or punishment CS, the DA (LHb) neurons exhibit a phasic dip (peak) activity upon CS. The DA (LHb) neurons show a base line in response to nonreward or punishment outcome but a phasic peak (dip) in response to the reward outcome. These phasic activities of DA and LHb neurons signal the uncertainty to the downstream of the neural system, but how the phasic activities arise from the local circuit is a challenge. Previous theoretical researches have applied the temporal difference learning and neural circuit model to investigate the phasic activity of DA neurons. However, the phasic activity of LHb neurons has not been taken into account. Considering that the phasic activity of LHb is opposite to that of DA neurons and there are connections between LHb and DA neurons, the influence of LHb should be considered. Here, we proposed a local circuit model to explore this issue. The response of DA neurons are controlled by an excitatory pathway (through striatum and PPTN) and an inhibitory pathway (through striosomes), which is similar to the previous models. The inhibitory pathway through striatum, ventral palladium, border of globus palladium (GPb) and the excitatory pathway through striosomes determine the responses of LHb neurons. The LHb neurons also innervate DA neurons through rostral medial tegmentum (RMTg). The simulations reproduce experimental observations on the phasic activation of neuron groups given reward and nonreward CS with or without reward outcomes: (i) A shift of DA and LHb neurons' responses from outcome to CS. (ii) The phasic activities of DA (LHb) neurons. The DA (LHb) neurons exhibit a phasic peak (dip) upon reward CS, and a base line in response to reward outcome but a phasic dip (peak) if the reward is omitted. By contrast, the DA (LHb) neurons exhibit a phasic dip (peak) upon nonreward CS or punishment CS, and a base line in response to the US but a

phasic peak (dip) if a reward occurs or the punishment US is omitted. (iii) The phasic activities of GPb and RMTg neurons are similar to that of LHb neurons.

Disclosures: D. Wang: None. **H. Zhou:** None.

Poster

755. Reward: Dopamine II

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Topic: F.03. Motivation and Emotion

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Title: Dopamine D2-receptor blockage stabilizes orbitofrontal reward representations

Authors: *T. KAHNT[1], S. C. WEBER[1], H. HAKER[2], T. W. ROBBINS[3], P. N. TOBLER[1]; [1]Dept. of Economics, UZH, Zurich, Switzerland; [2]ETH Zurich, Zurich, Switzerland; [3]Dept. of Psychology, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Prefrontal reward representations are critical for goal-directed behavior. The stability of prefrontal representations has been suggested to be modulated by dopamine. A current two-state model suggests that the stability of prefrontal network representations depends on the ratio of D1/D2 receptor activation. A D2-dominated state would allow for multiple but weak representations, whereas a D1-dominated state would favor few but stable representations. Here, we tested this model in the context of reward representations in the human orbitofrontal cortex (OFC) using dopamine receptor-specific pharmacology and multivoxel pattern-based functional magnetic resonance imaging (fMRI). Before performing a non-instrumental outcome-prediction task, human subjects received either 400 mg of the D2-receptor antagonist amisulpride (N = 27) or a placebo (N = 24) in a double-blind fashion. Behavior did not differ between groups, allowing a comparison of neural reward representations independent of potentially confounding differences in behavior. Using a searchlight decoding approach, we estimated the strength of reward representations in the OFC. In line with the model, we found that dopamine D2-receptor blockage significantly ($p < 0.05$, FWE whole brain corrected) strengthened distributed reward representations in the medial OFC. Moreover, we found a significant ($p < 0.05$, FWE whole brain corrected) dose-dependent increase in functional connectivity between the medial OFC and

the inferior parietal sulcus (IPS), suggesting that dopamine stabilizes prefrontal network representations by enhancing frontoparietal coupling. Our results show that dopamine D2 activation stabilizes prefrontal representations and are in line with the idea that dopamine gates the afferent input to the prefrontal cortex, thereby facilitating robust network representations.

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Poster

755. Reward: Dopamine II

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Topic: F.03. Motivation and Emotion

Support: MH093672-03S1

Title: Upregulation of dopamine D2 receptors in the nucleus accumbens indirect pathway enhances motivation

Authors: *E. F. GALLO, B. FENG, J. JAVITCH, C. KELLENDONK;
Psychiatry, Columbia Univ., NEW YORK, NY

Abstract: Dopamine signaling in the nucleus accumbens (NAc) plays a central role in the processing of reward-related information and in goal-directed behavior. Medium spiny neurons (MSNs), the main output cells of the NAc, typically belong to either the direct or indirect projection pathways, which differ in their respective enrichment of D1 and D2 dopamine receptors (D1Rs and D2Rs). Alterations in striatal D2R availability have been reported in several disorders that are characterized by motivational abnormalities, including schizophrenia, ADHD, and drug addiction. However, whether D2R levels are causally related to alterations in motivation is not fully understood. Recent evidence from our group demonstrated that viral-mediated D2R overexpression in the adult mouse NAc enhances motivation, but it remained unclear which neuronal population mediated this effect. Therefore, we selectively overexpressed D2Rs in the NAc indirect pathway (D2-MSNs) by injecting a conditional adeno-associated virus encoding D2R into the NAc of D2-Cre transgenic mice. We then tested the effect on motivation using a progressive ratio x2 (PRx2) schedule of reinforcement, where the lever-pressing requirement doubled with each reward. First, we found that D2 overexpression does not affect the initial operant learning in continuous reinforcement or fixed interval schedules compared to

control D2-Cre mice injected with a GFP virus. When tested on a PRx2 schedule, D2R-overexpressing mice pressed significantly more than controls (750.6 ± 114.7 vs 425.6 ± 73.27, $p<0.05$, n=7-8/group). In addition, the D2-overexpressing mice earned more rewards and continued to respond later than controls. We next tested the animals in a simple random ratio (RR) schedule and in a concurrent choice RR, where they were presented a freely-available, less preferred food (standard chow) while lever-pressing for a preferred food (milk). D2R-overexpressing mice showed significantly increased lever pressing compared to GFP-expressing mice at the highest ratios in the non-concurrent task. When chow was made available, both groups reduced their pressing for milk, but the D2R-overexpressing mice continued to press significantly more than controls, especially at lower ratios. These results indicate that postsynaptic D2R upregulation in in the indirect pathway of the adult NAc increases motivation. Moreover, the data suggest that the effect on motivation previously reported by our group is mediated primarily by D2R overexpression in D2-MSNs, raising the possibility that augmenting D2R levels or signaling in a cell-selective manner could be useful in the treatment of motivational dysregulation.

Disclosures: **E.F. Gallo:** None. **B. Feng:** None. **J. Javitch:** None. **C. Kellendonk:** None.

Poster

755. Reward: Dopamine II

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Topic: F.03. Motivation and Emotion

Support: R01-DA025634

Title: Central glucagon-like peptide-1 receptor activation mediates lithium chloride-induced dopamine suppression in the nucleus accumbens

Authors: *S. M. FORTIN, J. J. CONE, M. F. ROITMAN;
Univ. of Illinois At Chicago, Chicago, IL

Abstract: Unconditioned rewarding stimuli evoke increases in dopamine concentration in the nucleus accumbens (NAc). Furthermore, phasic dopamine responses to stimuli are plastic and appear to track hedonic value. Using a classical conditioned taste aversion paradigm, we have shown that once paired with injection of the malaise-inducing agent lithium chloride (LiCl), intraoral infusions of sucrose (which are normally rewarding and evoke phasic increases in

dopamine in the NAc) will suppress phasic dopamine signaling upon reexposure to the sucrose taste. While the conditioned effects of the aversive stimulus LiCl on phasic dopamine release patterns have been investigated, the unconditioned effects of the drug are unknown. Here we use fast-scan cyclic voltammetry to measure phasic dopamine increases in the NAc resulting from electrical stimulation of dopamine cell bodies in the ventral tegmental area (VTA). Peripherally administered LiCl rapidly and robustly suppressed phasic dopamine release in the NAc core (-35.35 +/- 7.9 vs 5.97+/- 10.0 % change from baseline for LiCl vs vehicle injection i.p.). As some behavioral effects of LiCl appear to be mediated through glucagon-like peptide-1 receptor (GLP-1R) activation, we hypothesized that the suppression of phasic dopamine by LiCl is GLP-1R dependent. Indeed, peripheral pretreatment with the GLP-1R antagonist exendin-9 (Ex-9) potently attenuated the LiCl-induced suppression of dopamine (-8.37 +/- 3.5% change). Pretreatment with GLP-1R antagonist did not, however, affect the suppression of phasic dopamine release by the kappa opioid receptor agonist, salvinorin A - supporting a selective effect of GLP-1 receptor stimulation in LiCl-induced dopamine suppression. We extended this work by administering Ex-9 to the lateral ventricle and replicated the attenuation in dopamine suppression by peripheral LiCl (-4.41 +/- 5.0 vs -31.24 +/- 3.0 vs 0.62 +/- 7.0 % change for vehicle (lateral i.c.v.)/vehicle (i.p.), vehicle/LiCl, and Ex-9/LiCl groups, respectively). We, therefore, highlight a critical role for central GLP-1R activation in the dramatic decrease in excitability of midbrain dopamine neurons by the unconditioned aversive stimulus LiCl.

Disclosures: S.M. Fortin: None. **J.J. Cone:** None. **M.F. Roitman:** None.

Poster

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Program#/Poster#: 755.07/UU83

Topic: F.03. Motivation and Emotion

Title: Microinjections of a dopamine D1 receptor antagonist into the ventral tegmental area blocks the expression of cocaine conditioned place preference in rats

Authors: *E. J. PAWUL[1], M. MANUSZAK[2], D. ARASTEHMANESH[2], R. RANALDI[1,2]; [1]The Grad. Ctr. , City Univ. of New York, New York, NY; [2]Psychology, Queens College/ CUNY, Flushing, NY

Abstract: Stimulation of dopamine (DA) D1 receptors in the ventral tegmental area (VTA) is involved in primary rewards. In the current study we investigated whether VTA D1 receptor

stimulation likewise plays a role in mediating the rewarding effects of cocaine-associated stimuli, using the cocaine conditioned place preference (CPP) paradigm. Rats were prepared with cannulae so as to allow microinjections in the VTA and later conditioned to a cocaine-associated environment using the CPP paradigm. Prior to each conditioning session rats were injected with either saline or cocaine (10 mg/kg, intraperitoneally) and then placed in one of the two sides of the CPP apparatus. Sessions lasted thirty minutes a day over a period of eight days, such that rats alternated daily between consistently experiencing cocaine in one side and saline in the other. On the test day, which was conducted one day after conditioning, rats were given bilateral microinjections of one of four doses of the D1 antagonist, SCH 23390, (0, 2, 4 or 8 μg/0.5 μl) directly into the VTA and allowed free access to both sides of the apparatus. Preference for either side was measured as time spent in each side and compared to the same measures taken before conditioning. The D1 antagonist, when injected into the VTA, produced a dose-related significant reduction in the preference for the cocaine-paired side compared to vehicle. When injected just dorsal to the VTA, the D1 antagonist produced no effect. These data suggest that the expression of cocaine conditioned place preference requires stimulation of VTA D1 receptors and, as such, are the first to suggest a role for VTA dendritically released DA in cocaine-, or other reward-, related learning.

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Poster

755. Reward: Dopamine II

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Program#/Poster#: 755.08/UU84

Topic: F.03. Motivation and Emotion

Support: Wellcome Trust

Title: Monkey choice behavior is biased by phasic stimulation of channelrhodopsin expressing dopamine neurons

Authors: *W. R. STAUFFER[1], A. LAK[1], A. YANG[2], M. BOREL[1], O. PAULSEN[1], E. BOYDEN[2], W. SCHULTZ[1];
[1]Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom;
[2]McGovern Inst., MIT, Boston, MA

Abstract: The phasic responses of dopamine neurons encode reward prediction errors, the difference between predicted and received reward. Electrical and optical stimulation of dopamine neurons in rodents has demonstrated that phasic activation can act as a reward. However, the application of optogenetic technologies to primate behavior has been limited by technical challenges, including the limited ability to specify expression to particular cell-types. Here we simultaneously injected two viral vectors to specifically label dopamine neurons in mouse and macaque monkey midbrain. The first vector was an AAV9 that expressed Cre-recombinase under the control of the Tyrosine Hydroxylase (TH) promoter. The second vector was a standard double-floxed channelrhodopsin (ChR2) vector (pAAV5-Ef1a-DIO-hChR2(H134R)-EYFP). The coordinates for the mouse injections were taken from a standard stereotactic atlas (Franklin and Paxinos, 2007), whereas the monkey injections were guided by coordinates of electrophysiologically localized and identified dopamine neurons. Between 30 and 50% of TH labeled dopamine neurons near the injection sites expressed ChR2 in mice and monkey. Importantly, the specificity of expression was greater than 90%. The electrophysiological functionality of the expressed ChR2 was verified in brain slices made from the mice. We characterized the behavioral effect of channelrhodopsin stimulation during value-based choice. Monkeys made choices between different options that offered optical stimulation, electrical stimulation or juice rewards. The animal learned to choose the cue that provided optical stimulation, and was indifferent between 200 ms of optical stimulation and 0.1 ml of juice. The value-like effect of optical stimulation was smaller than that of electrical stimulation, although this difference could be due to several factors, including the non-specificity of electrical stimulation, the level of expression, and the limited spread of delivered light. These results demonstrate the efficacy of a two-virus approach to gain cell-type specificity in a monkey, and that phasic stimulation of midbrain dopamine neurons is sufficient to bias value-based choices.

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Poster

755. Reward: Dopamine II

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Topic: F.03. Motivation and Emotion

Support: SFARI

NIDA

Title: Optogenetic dissection of the mesohabenular projection system in mice

Authors: S. LAMMEL, *E. STEINBERG, C. FÖLDY, N. R. WALL, K. BEIER, R. C. MALENKA;
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Abstract: The lateral habenula (LHb) has been shown to strongly inhibit ventral tegmental area (VTA) dopamine (DA) neurons and is emerging as a structure capable of conveying information about rewarding and aversive events. Notably, the VTA also sends a robust reciprocal projection to the LHb. Using a combination of anatomical tracing techniques, optogenetics and electrophysiology we investigated the neurochemical identity and function of VTA neurons projecting to LHb. Our results demonstrate that the vast majority (~97-99%) of these neurons are non-dopaminergic (i.e. TH-immunonegative). Surprisingly, following injection of Cre-dependent AAV viral vectors expressing eYFP or channelrhodopsin-2 (ChR2)-eYFP into the VTA of TH:Cre mice we observed substantial transgene expression in LHb terminals. In contrast, similar injections into DAT:Cre mice revealed minimal terminal labeling in the LHb. Subsequent retrograde tracing experiments revealed that all eYFP-expressing LHb-projecting neurons in the VTA of TH:Cre mice were immunonegative for TH (36/36 cells, n=2 mice). Because of this inconsistency, we performed an extensive anatomical, molecular and functional characterization of prominent DA transgenic mouse driver lines (TH:Cre, TH:GFP and DAT:Cre) and demonstrate that mice under the control of TH, but not DAT, promoter exhibit significant transgene expression in TH-immunonegative (i.e., non-DAergic) neurons within and around VTA nuclei. Importantly, a large number of eYFP-positive cells in the ventral midbrain of TH:Cre mice lacked DAergic markers but showed molecular and electrophysiological properties which previously have been observed in VTA GABAergic neurons. Cre-dependent ChR2-eYFP expression in the VTA of GAD2:Cre or VGlut2:Cre mice revealed substantial terminal labeling in the LHb, suggesting that VTA GABAergic and glutamatergic neurons project to the LHb. Indeed, light pulses that selectively stimulated GAD2:Cre ChR2 fibers in the LHb evoked inhibitory postsynaptic currents (-370.4±94.1 pA, n=9 cells) that were inhibited by the GABA-A receptor antagonist picrotoxin (-25.3±5.6 pA, n=5 cells) in ex-vivo brain slice patch-clamp recordings. Currently, we are exploring the behavioral role of GABAergic and glutamatergic projections to the LHb.

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Poster

755. Reward: Dopamine II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 755.10/UU86

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA035943

Title: Distinct midbrain dopamine neuron subpopulations contribute to cue attraction, psychomotor activation, and reinforcement

Authors: *B. T. SAUNDERS, P. H. JANAK;
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Abstract: The specific nature of dopamine's (DA) role in motivated behavior is a matter of debate. This is due in part to the anatomical complexity of the system, where neurons in the more medial ventral tegmental area (VTA) project largely to the prefrontal cortex and nucleus accumbens, while neurons in the more lateral substantia nigra (SN) project to the dorsal striatum. To parse the contribution of these distinct subpopulations to different facets of appetitive motivation and learning, we targeted expression of channelrhodopsin to dopaminergic neurons in TH::cre rats. Pavlovian Conditioning: TH::Cre+/- rats first received Pavlovian training, in which blue laser (473nm) was delivered unilaterally to either the VTA or SN, independent of behavior. For PAIRED rats, this stimulation was predicted by the presentation of external cues (light + tone), while for UNPAIRED rats cues and stimulation were explicitly unassociated. We found that, across training, different conditioned responses to the Pavlovian cues emerged as a function of laser stimulation target within the midbrain. PAIRED rats receiving the most medial VTA stimulation developed cue-light approach behavior, suggesting the cue had become attention grabbing and attractive. More lateral VTA stimulation produced locomotion in response to the cue, expressed as rotation contralateral to the hemisphere of laser delivery. By the end of training, this cue-evoked rotation occurred before laser onset, suggesting that the cue spurred psychomotor activation. Cues predictive of SN laser delivery elicited only conditioned rotation. Importantly, UNPAIRED rats did not develop these conditioned responses, even though they received the same number of laser stimulations. Conditioned Reinforcement: Following Pavlovian training, rats were given the opportunity to lever press for cue presentations in the absence of laser delivery. Rats robustly responded for cues that had been paired with VTA, but not SN, DA neuron stimulation. Intracranial Self Stimulation: Finally, rats were allowed to nose poke for brief laser pulses. This self-stimulation behavior was similarly robust regardless of laser target site in the VTA and SN. Together, these results demonstrate that anatomically distinct DA neuron subpopulations control the attribution of motivational value to neutral cues to spur attraction, psychomotor activation, and support conditioned reinforcement, while the ability of DA neurons to directly reinforce actions is relatively consistent throughout the midbrain. This

points to circuit-level “rules” by which DA neurons contribute to a diverse set of motivational processes.

Disclosures: B.T. Saunders: None. **P.H. Janak:** None.

Poster

755. Reward: Dopamine II

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Topic: F.03. Motivation and Emotion

Support: NIH Grant DA025634

Title: Chemogenetic inhibition of mesolimbic dopamine is insufficient to condition taste aversion

Authors: *S. M. CONWAY, S. M. FORTIN, C. G. SINON, J. E. MCCUTCHEON, M. F. ROITMAN;
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Abstract: Dopamine (DA) projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) are widely recognized as playing a role in aspects of reward, but their role in aversion is less clear. Recent work has demonstrated that rodents avoid areas associated with selective inhibition of DA neurons in a manner consistent with a conditioned place aversion. We investigated whether suppression of NAc DA signaling would be sufficient to condition another type of learned aversion - a conditioned taste aversion to a normally appetitive taste. We injected the VTA of transgenic rats expressing Cre recombinase in tyrosine hydroxylase expressing cells (TH:Cre) and wildtype littermates (TH:WT) with Cre-dependent virus to express the inhibitory chemogenetic receptor, hM4D(Gi) (a Designer Receptor Exclusively Activated by Designer Drug, DREADD). Thus, systemic administration of the DREADD ligand, clozapine-N-oxide (CNO), should suppress DA neuron excitability and subsequent NAc DA release in TH:Cre but not TH:WT rats. To validate the function of DREADD, TH:Cre and TH:WT rats received two cocaine-induced locomotor tests with pretreatment of either CNO or saline. Only TH:Cre rats exhibited significantly lower cocaine-induced locomotion when pretreated with CNO versus saline ($p=0.02$). A separate set of locomotor tests indicated no effect of CNO on spontaneous locomotion. Additionally, fast-scan cyclic voltammetry was employed to determine CNO effects on electrically-evoked DA release in the NAc. CNO injection significantly suppressed evoked

DA release only in TH:Cre rats (31% decrease from baseline, 45min post-CNO injection). To determine if suppressed DA signaling is sufficient to condition a taste aversion, three sessions of brief intra-oral infusions of saccharin were administered to initially naïve TH:Cre and TH:WT rats. Immediately following each session, rats received either injections of CNO, the malaise-inducing agent LiCl, or saline. Rats were then given overnight access to saccharin and water in a two-bottle preference test. Only the LiCl-paired group exhibited a conditioned taste aversion - preferring water to saccharin whereas CNO-paired TH:Cre rats exhibited a clear preference for saccharin similar to that of CNO- and saline-paired TH:WT rats. Taken together, the results indicate that while inhibitory DREADD activation suppresses DA signaling and cocaine-induced locomotion, it is insufficient to condition a taste aversion; the data suggest that while suppressed DA signaling is sufficient to condition place aversions, additional neural circuits are required for aversions to taste.

Disclosures: S.M. Conway: None. **S.M. Fortin:** None. **C.G. Sinon:** None. **J.E. McCutcheon:** None. **M.F. Roitman:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01MH091119

Title: Vesicular co-release of GABA with glutamate in the lateral habenula: Implications for depression

Authors: *S. SHABEL[1], C. PROULX[1], J. PIRIZ[2], R. MALINOW[1];
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Abstract: The lateral habenula (LHb), a key regulator of monoaminergic brain regions, is activated by negatively-valenced events and its hyperactivity is associated with depression. While recent studies suggest that enhanced excitatory (glutamatergic) input to the LHb may be linked to depression, little is known about inhibitory (GABAergic) transmission. Here we show that GABA is co-released with its functional opponent, glutamate, from long-range basal ganglia inputs (which signal negative events) to limit LHb activity in rodents. Electrophysiological and

ultrastructural data indicate that GABA and glutamate are co-released from individual vesicles. Interestingly, at this synapse, co-release of GABA is reduced in a model of depression and enhanced by antidepressant treatment. These findings show that GABA and glutamate co-release controls LHb activity and that regulation of this remarkable form of transmission may be important for determining the impact of negative life events on mood and behavior.

Disclosures: **S. Shabel:** None. **C. Proulx:** None. **J. Piriz:** None. **R. Malinow:** None.

Poster

755. Reward: Dopamine II

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JPB Foundation

Title: Identifying inputs to dopamine neurons in the dorsal raphe and establishing their functional role

Authors: *G. A. MATTHEWS[1], C. M. VANDER WEELE[1], R. E. THOMAS[1,2], N. GOLAN[1,2], I. R. WICKERSHAM[3], K. M. TYE[1];
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Abstract: The midbrain dopamine system plays a fundamental, evolutionarily-conserved role in regulating behaviors including motor control, reward, motivation, and emotional processing. The

most frequently studied midbrain dopamine neurons are those located within the substantia nigra (SN) and ventral tegmental area (VTA). However, a lesser known and understudied population of dopamine neurons reside within the dorsal raphe nucleus (DRN) and ventrolateral periaqueductal grey (vlPAG). Accumulating evidence suggests these neurons are anatomically and functionally distinct from SN and VTA dopamine neurons. Specifically, in contrast to the VTA, the DRN/vlPAG dopamine neurons provide the majority of the dopamine input to the central amygdala and bed nucleus of the stria terminalis, but very little to the nucleus accumbens and prefrontal cortex. However, the function of this population of dopamine neurons remains poorly understood. We are using a combination of rabies virus-mediated monosynaptic tracing and channelrhodopsin (ChR2)-assisted circuit mapping to establish the upstream inputs to this dopamine subpopulation. For monosynaptic tracing we injected a Cre-dependent adeno-associated virus (AAV) encoding the avian TVA viral receptor and the rabies virus glycoprotein into the DRN/vlPAG of TH-Cre mice. This was followed a week later by the modified rabies virus (pseudotyped with an avian virus envelope protein; EnvA) in order to restrict expression to dopamine neurons. Using this approach we identified several upstream sites which appear to provide strong input onto these dopamine neurons. We have confirmed a direct input from these sites by expressing AAV5-CaMKIIα-ChR2-eYFP in a single upstream site and recording optically-evoked synaptic currents in DRN/vlPAG dopamine neurons in brain slices. Additionally, we are examining the effect of activating this dopamine population *in vivo* to determine the role of these neurons in behavior. We anticipate that this will extend our understanding of the midbrain dopamine system and help tease apart its heterogeneity.

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Poster

755. Reward: Dopamine II

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Support: NIH Grant R01 MH094489 (PDS)

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Title: Paradoxical excitation of VTA neurons during electrical stimulation of the fasciculus retroflexus in rat sagittal brain slices

Authors: *P. L. BROWN[1], P. D. SHEPARD[2];
[1]Program in Neurosci., [2]Dept. of Psychiatry and Maryland Psychiatric Res. Ctr., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: The lateral habenula (LHb), a phylogenetically conserved, epithalamic area, is activated by aversive stimuli and reward omission. Electrical stimulation of the LHb elicits a population wide inhibition of midbrain dopamine (DA) cell activity, a phenomenon that is believed to be a neuronal representation of reward prediction error. While the majority of midbrain DA neurons are inhibited by aversive stimuli, some are excited, suggesting that they are encoding environmental salience rather than prediction errors. A minority of glutamatergic efferents from the lateral habenula directly target dopamine and non-dopamine neurons in the ventral tegmental area (VTA). In the present study, we tested whether activation of habenular efferents has demonstrable excitatory effects on VTA neurons in rats using a para-sagittal slice preparation containing habenular efferents (fasciculus retroflexus), the VTA, and the RMTg. Individual VTA neurons were recorded using whole cell patch clamp, filled with neurobiotin for subsequent visualization and classified as DA or non-DA by immunochemical visualization of tyrosine hydroxylase. As a group, dopamine neurons (n=52) had a significantly slower firing rate, longer time constant, and wider spike waveform than non-dopamine neurons (n=63). Single pulse stimulation (150 μs, 10-50 V) of the fasciculus retroflexus activated 48% of DA and 51% of non-DA neurons in the VTA. Relatively few neurons (7%) were inhibited. The increase in firing rate was longer in duration and later in onset for VTA DA neurons compared to VTA non-DA neurons. Excising the area posterior to the third cranial nervewhich included the RMTg, did not alter the response of VTA neurons to fr stimulation. Under current clamp conditions, fr stimulation evoked EPSPs with a nearly constant onset latency, indicative of a monosynaptic connection. Bath application of DNQX and APV (10 and 50 μM; n=3) blocked the stimulation induced increase in firing rate in non-DA neurons, consistent with the glutamatergic nature of this projection. These data are consistent with the finding that some VTA neurons are activated by aversive stimuli, and suggest that the LHb is a potential source of such activation.

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Poster

755. Reward: Dopamine II

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UIC Dean's Scholar Fellowship

Title: Phasic dopamine signaling tracks the reward value of sodium based on physiological state

Authors: *J. J. CONE[1], S. M. FORTIN[1], J. E. MCCUTCHEON[2], M. F. ROITMAN[1]; [1]Psychology, Univ. of Illinois At Chicago, Chicago, IL; [2]Cell Physiol. and Pharmacol., Univ. of Leicester, Leicester, United Kingdom

Abstract: While it is widely recognized that rewarding stimuli evoke phasic increases in dopamine activity, there is less agreement on how the mesolimbic system encodes aversive stimuli. We have previously shown that a primary rewarding taste stimulus increases, while an aversive taste or a taste that is initially rewarding but acquires aversive properties through conditioning, suppresses phasic dopamine release in the nucleus accumbens (NAc). Here, we further challenged the idea that rewarding and aversive stimuli are differentially encoded by phasic dopamine signaling. We administered brief intra-oral infusions of a concentrated (0.45M) NaCl solution while measuring resultant changes in NAc dopamine signaling using fast-scan cyclic voltammetry. Prior to recording, we depleted rats of sodium with furosemide and maintained them on sodium-deficient chow and water for 24 hours (deplete; n=4). Control animals were given vehicle injections and given standard chow and water (replete; n=6). A 0.45M NaCl solution is normally avoided and intra-oral delivery elicits aversive taste reactivity. However, when rats are made sodium deplete, a sodium appetite develops and the same NaCl solution now evokes appetitive taste reactivity and positively reinforces behavior. If phasic dopamine signaling differentially encodes rewarding and aversive stimuli, then 0.45M NaCl should evoke very different patterns of NAc dopamine depending on physiological state (e.g. sodium balance). Indeed, phasic dopamine evoked by intraoral 0.45M NaCl tracked the rewarding value of salt in a state-dependent manner. Intra-oral NaCl infusions evoked a robust increase in dopamine (26.08 +/- 3.6nM) in deplete rats, whereas the same solution suppressed dopamine in replete rats (-2.37 +/- 0.83nM). Notably, dopamine evoked by intra-oral NaCl was evident on the first trial in deplete rats; indicating the dopamine response is experience independent. Post-recording consumption of a 0.45M NaCl solution confirmed a sodium appetite only in deplete rats. A second group of deplete rats (n=5) received 0.45M NaCl with 100µM amiloride - which blocks amiloride-sensitive lingual sodium channels and disrupts detection of sodium in solution. Amiloride significantly attenuated the dopamine response to 0.45M NaCl (7.66 +/- 2.6nM), indicating that it is the taste of sodium that drives the dopamine response to NaCl infusions in deplete rats. Taken together, we show that the dopamine response to 0.45M NaCl is dependent on an interaction between taste and physiological state. These data present a strong argument for differential encoding of reward and aversion by phasic dopamine concentration changes in the NAc.

Disclosures: **J.J. Cone:** None. **M.F. Roitman:** None. **S.M. Fortin:** None. **J.E. McCutcheon:** None.

Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: AA014925

Title: Optical stimulation of nucleus accumbens shell-projecting basolateral amygdala neurons suppresses Pavlovian cue-conditioned alcohol seeking and unconditioned homecage alcohol drinking

Authors: ***Z. MILLAN**, P. H. JANAK;
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Abstract: The nucleus accumbens shell (AcbSh) strongly inhibits feeding during satiety, reward seeking in the presence of an unrewarded cue, and drug seeking following its extinction. Understanding the mechanisms that promote suppressive control over motivated behavior has important implications for pathologies such as drug addiction, which is characterized by a significant loss of control over drug use. Here we used an optogenetic approach to examine basolateral amygdala (BLA) projections that target AcbSh and to test whether stimulation of these AcbSh-projecting BLA terminals might be sufficient to suppress conditioned alcohol-seeking in the presence of an EtOH-predictive cue. Rats received intermittent homecage access to EtOH (15%v/v) and subsequently were trained to acquire Pavlovian conditioned alcohol seeking given repeated trials of a 10s auditory cue (CS) paired with EtOH (15% v/v) delivery. On test, rats were assessed for cue-triggered alcohol seeking (port entries) in the presence of both non-reinforced and reinforced presentations of the CS. We found that 5s stimulation of BLA terminals in AcbSh at the time of CS onset abolished conditioned port entries under non-reinforced extinction conditions. On subsequent CS-reinforced tests, optical stimulation of BLA terminals in AcbSh reduced cue-triggered alcohol seeking when all events -- stimulation, EtOH delivery and CS onset -- co-occurred. Conversely, when alcohol was delivered during the latter portion of the CS interval, coinciding with the offset of the optical stimulation, rats maintained their ability to port entry within the interval of the cue, although at a delayed latency to respond. This latter finding suggests that the impact of stimulation on conditioned port entries is well-

timed to the duration of stimulation. Finally, we examined whether the inhibitory effect of BLA terminal stimulation in AcbSh was specific to cue-conditioned behavior or whether it extended to unconditioned EtOH drinking under brief homecage access conditions. Rats were placed on a 15 min homecage access regime prior to stimulation test. We found that stimulation prevented unconditioned EtOH drinking under a two-bottle choice test. Together, these findings suggest that recruitment of a BLA-AcbSh pathway is sufficient to inhibit alcohol-reinforced conditioned and unconditioned behavior, and may be involved in maintaining control over alcohol-motivated behavior.

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Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: DA 023206

DA030379

DA 034856

DA035805

MH101147

DA036303

Title: Bi-directional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections

Authors: *Y. Y. MA[1], B. R. LEE[2], X. WANG[1], C. GUO[1], L. LIU[3], Y. LAN[4], J. J. BALCITA-PEDICINO[1], R. CUI[3], S. R. SESACK[1], Y. SHAHAM[5], O. M. SCHLÜTER[6], Y. H. HUANG[1], Y. DONG[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Allen Institue, seattle, WA; [3]Sch. of life science, Northeastern Normal Univ., Changchun, China; [4]Physiol. and Pathophysiology, Yanbian Univ., Yanji, China; [5]NIH, Baltimore, MD; [6]European Neurosci. Inst., Grisebachstr, Germany

Abstract: Extensive efforts have been devoted to understand drug-induced neuroadaptations that promote addiction, with the goal of reversing these alterations for therapeutic benefit. However, endogenous anti-addiction processes may also be induced by drug exposure, and these self-correcting mechanisms have not been sufficiently explored and exploited. Drug-induced alterations in glutamatergic synaptic transmission to the nucleus accumbens (NAc) have been critically implicated in drug seeking, relapse, and other addiction-associated behaviors. The deep layers of the medial prefrontal cortex (mPFC) provides a major glutamatergic input to the NAc, with infralimbic PFC (IL) preferentially projecting to the NAc shell (NAcSh) and prelimbic PFC (PrL) preferentially projecting to the NAc core (NAcCo). Here, we show that although both of these glutamatergic projections undergo silent synapse-based remodeling after cocaine self-administration, the remodeling of IL-to-NAcSh projections functions to dampen cue-induced cocaine seeking after cocaine withdrawal. Silent synapses are thought to be immature excitatory synapses that only express NMDA receptors (NMDARs) with no stable AMPA receptors (AMPARs). De novo generation of silent synapses may create new synaptic contacts, and subsequent unsilencing/maturation of these silent synapses by recruiting new AMPARs may not only strengthen the affected neurocircuits, but also create new forms of informational flow. By characterizing the progressive remodeling of IL-to-NAcSh projection via generation and subsequent maturation of silent synapses after cocaine exposure, the present results reveal an endogenously-induced mechanism that may counteract the development of cocaine addiction and reduce relapse.

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Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: Swedish Research Council K2012-77PK-22164-01-2

Title: Evidence for long-range projecting parvalbumin-expressing (PV+) neurons in the lateral habenula that directly target dopaminergic and serotonergic neurons

Authors: *L. POZZI, I. POLLAK DOROCIC, I. LAZARIDIS, Y. XUAN, M. CARLEN, K. MELETIS;
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Abstract: The lateral habenula (LHb) has been implicated in motivated behavior as well as in depressive disorders based on its control of the dopamine and serotonin systems. We focused on the expression of the GABAergic inhibitory calcium binding protein parvalbumin (PV) in the LHb. To selectively target PV+ neurons in LHb, we introduced a Cre-inducible viral construct coding for channelrhodopsin-2 fused to an enhanced red fluorescent protein (AAV5-DIO-Chr2-mCherry) unilaterally into the LHb of PV-Cre adult mice. Four weeks after the injections we found mCherry-PV+ neurons in the LHb and mCherry-positive fibers in the medial VTA and in both median (MR) and dorsal (DR) raphe nuclei. To directly identify if PV+ neurons in LHb have long range projections that directly contact dopaminergic or serotonergic neurons, we used transgenic mouse lines expressing Cre recombinase only in dopaminergic (Syt17-Cre mice) or serotonergic (SERT-Cre mice) neurons together will cell type-specific rabies tracing. We found that the main target of PV+ long range projections was the MR (24% of total GFP+ cells in the LHb also coexpressing PV), followed by the DR (3,7%) and the VTA (3%). Our results provide the first direct evidence of monosynaptic inputs between PV+-long-range projection from LHb onto dopaminergic cells in VTA and serotonergic cells in DR and MR. Taken the importance of LHb as a modulatory nucleus of the dopaminergic and serotonergic system the definition of its connectivity and function will provide valuable insights into the understanding of both motivated behavior and depressive disorders.

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Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: NARSAD

Title: The LHb regulates the dorsal raphe nucleus through a direct and an indirect pathway

Authors: *C. PROULX, C. MOLINA, R. MALINOW;
Neurosciences, Univ. of California, San Diego (UCSD), La Jolla, CA

Abstract: The lateral habenula (LHb) is an epithalamic structure receiving inputs from the forebrain limbic system and projects to aminergic midbrain structures, such as the dorsal raphe nucleus (DRN). Interest in the biological role of LHb increased in the past couple years when its importance as an anti-reward center was proposed. The LHb has been demonstrated to be a key player of the reward system mainly by inhibiting dopaminergic centers. However, the nature and potential regulation of the DRN, the main serotonergic center, by the LHb is poorly understood. To explore how the LHb regulates DRN activity, we injected in the LHb of rats an AAV driving expression of the channelrhodopsin2 (AAV-ChR2). Three weeks later, we obtain whole-cell voltage-clamp recording from DRN neurons and LHb terminals were activated with brief light pulses. eEPSCs (>10pA) responses were measured from 50 out of 55 DRN neurons; only 5 eIPSCs (>10pA) could be detected. 50% of recorded, biocytin-filled DRN neurons were positive for serotonergic marker (11/22). This suggests that direct projections from LHb mainly result in an overall activation of the DRN. Previous findings from *in vivo* electrical stimulation suggested that the LHb has an inhibitory impact on DRN activity. To test if RMTg projects to and inhibit the DRN, AAV-ChR2 was injected in RMTg and whole-cell voltage-clamp recordings were obtained from DRN neurons. No eEPSCs could be detected while large eIPSCs were measured from all recorded DRN neurons (n=8). To confirm these *in vitro* results, we injected AAV-ChR2 in LHb and 4 weeks later, single unit recording were obtained in DRN of anesthetized rats. 20Hz stimulation of LHb somata or LHb terminals in DRN mainly resulted in an increase in spiking frequency of DRN neurons. Interestingly, light-evoked activation (20Hz) of LHb terminals in RMTg decreased spiking frequency in DRN. To test the effect of direct activation of DRN by the LHb behaviorally, AAV-ChR2 was injected in LHb and optic fiber cannula were implanted above the DRN. In a 2-compartment shuttle box, pairing optogenetic stimulation of LHb terminals in DRN with one the two contexts, significantly induces avoidance of this context. In conclusion, *in vitro* and *in vivo* electrophysiological recordings show that excitatory axons from the LHb have a largely excitatory effect on DRN activity, contacting both serotonergic and non-serotonergic DRN neurons, while activation of the LHb-RMTg terminals largely inhibit DRN activity. Activation of the direct pathway is aversive.

Disclosures: **C. Proulx:** None. **C. Molina:** None. **R. Malinow:** None.

Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Title: Dissecting neural circuitry involved with rewarding stimulation of the supramammillary nucleus

Authors: *A. KESNER, S. IKEMOTO;
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Abstract: Andrew J. Kesner and Satoshi Ikemoto Behavioral Neuroscience Branch, National Institutes on Drug Abuse (NIDA), National Institutes of Health (NIH), Department of Health and Human Services, Baltimore, USA The supramammillary nucleus (SuM) is a small posterior hypothalamic nucleus situated just above the mammillary bodies. A prominent feature of the SuM is that it provides dense projections to the septo-hippocampal area. Past research on this nucleus primarily focused on its role as a signal integrator and active modulator for hippocampal theta rhythm activity. However, previous work in our lab has shown that rats learn to intracranially self-administer several pharmacological agents into the SuM - including AMPA, nicotine, and $GABA_A$ antagonist picrotoxin. These pharmacological data strongly suggest that general excitation of SuM neurons induces reward. In the present study, we used an optogenetic approach to further test the hypothesis that stimulation of SuM neurons is rewarding. We first confirmed this hypothesis using an adeno-associated virus (AAV) encoding *channelrhodopsin-2* under a *human synapsin-1* gene promoter in wild-type (C57/BL7) mice; where after 3 weeks of a channelrhodopsin-2 synthesis period, each mouse was placed in an operant conditioning chamber equipped with two levers, and given the opportunity to respond on the levers. Mice quickly learned to respond on the ("active") lever reinforced by photostimulation (473nm, 15 pulses at 25Hz) via the implanted optic fiber, while they responded little on the ("inactive") lever reinforced by no photostimulation. Mice do not reliably self-stimulate when the AAV-vector is injected into the SuM and optic fibers are placed in the mammillary bodies, just ventral to the SuM, or in the ventral tegmental area, just posterior/lateral to the SuM. This data clearly identifies the SuM as a brain region associated with reward processes. Next we used transgenic mice to target specific populations of neurons within the SuM. TH-Cre, vGat-Cre, and vGlut2-Cre mice were used to stimulate dopaminergic, GABAergic, or glutamatergic neurons, respectively. Only vGlut2-Cre mice reliably self-stimulate for cell body excitation in SuM. Lastly we used optogenetic terminal-stimulation to dissect which glutamatergic projections from SuM mediate self-stimulation behavior. Mice learned to respond for the stimulation of SuM glutamatergic neurons terminating in the lateral septum. Our findings suggests that SuM neurons are involved with reward signaling, and that this reward signaling may be mediated by SuM glutamtergic neurons synapsing in the lateral septum. This work was supported by NIDA/NIH.

Disclosures: **A. Kesner:** None. **S. Ikemoto:** None.

Poster

756. Motivation and Emotions: Reward Circuitry

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Support: CIHR Grant MOP 89758

NSERC RGPIN 261739-2008

Title: Sources of dopamine fibers in the paraventricular nucleus of the thalamus

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Abstract: The paraventricular nucleus of the thalamus (PVT) sends a dense projection to the nucleus accumbens, bed nucleus of stria terminalis, central nucleus of the amygdala, and the prefrontal cortex. These areas of the brain play a key role in regulating reward and motivation. Recent experimental evidence indicates that the PVT is involved in the modulation of drug seeking behavior and reward behavior in rats. It is well known that dopamine (DA) neurons in the ventral tegmental area (VTA; A10 group) through its projections to the nucleus accumbens are a key regulator of reward and motivation. Dopamine fibers have also been localized in the PVT and it is possible that DA from the VTA could influence behavior by acting at PVT. Previous studies that have attempted to identify the source of DA fibers in the PVT in the rat have been inconsistent in terms of whether the VTA is a source of these fibers. The PVT is a small nucleus which is bordered by the mediodorsal nucleus and the habenula, both of which receive DA projections from the VTA. The present study was done to re-examine this important question. Small iontophoretic injections of cholera toxin B (CTB) that were restricted to the PVT were used to retrogradely labeled tyrosine hydroxylase positive neurons that project to the PVT. Dopamine (tyrosine hydroxylase positive) neurons that were found to project to the PVT were scattered through different regions of the hypothalamus (A11, A13, A15 DA cell groups) and the periaqueductal gray. Double-labeled neurons were sometimes found in the VTA in cases with larger injections that spread outside the PVT, indicating that the areas outside the PVT may receive DA fibers from the VTA. We can conclude from these experiments that DA fibers in the PVT do not originate from VTA but from a heterogeneous population of DA neurons located in the hypothalamus and periaqueductal gray. The significance and function of DA in the PVT remains to be determined.

Disclosures: S. Li: None. **G.J. Kirouac:** None.

Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: R01MH093672

Title: Decreasing excitability of indirect-pathway medium spiny neurons acutely, but not chronically, leads to increased motivation in an endophenotypic model of schizophrenia

Authors: *F. D. DE CARVALHO[1], M. CAZORLA[2], M. SHEGDA[1], E. B. HOLZNER[3], M. J. F. LUNG[4], P. D. BALSAM[3], C. KELLENDONK[1];
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Abstract: The negative symptoms of schizophrenia respond poorly to medications. A transgenic mouse model of dopamine D2 receptor (D2R) overexpression in the striatum (D2R-OE) replicates the increased availability and activity of D2Rs observed in the striatum of patients. D2R-OE mice have a deficit in incentive motivation, which is a feature of the negative symptoms of schizophrenia. D2R-OE mice exhibit hyperexcitable striatal medium spiny neurons (MSNs) and altered connectivity in the basal ganglia. Specifically, they have increased density of "bridging collaterals", axon collaterals of direct-pathway MSNs that target the GPe and functionally bridge the two parallel pathways of the basal ganglia. These collaterals are highly plastic in the adult: their growth can be induced by increasing excitability of indirect-pathway MSNs in wild type mice, and they can be retracted in D2R-OE mice by decreasing excitability of these same neurons. Here, we used a pharmacogenetic approach to investigate the role of the bridging collaterals and MSN excitability on the motivation deficit of D2R-OE mice. The Gi-coupled synthetic receptor hM4D receptor gene was virally transferred into the striatum and subsequently activated acutely or chronically by administration of its synthetic ligand clozapine-N-oxide (CNO). We found that chronically decreasing excitability of MSNs led to retraction of bridging collaterals but did not rescue the motivation deficit of D2R-OE mice, whether or not mice were on CNO during behavior testing. However, acutely decreasing indirect-pathway MSN excitability led to increased motivation in both D2R-OE and control mice. These findings suggest that the bridging collaterals do not play a role in the motivation phenotype of D2R-OE mice. In addition, the motivation deficit in D2R-OE mice can be reversed by decreasing excitability in D2R-positive MSNs acutely but not chronically.

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Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: NIH F32 AA022290

Title: Ventral pallidum neurons encode reward-seeking in response to an instrumental incentive stimulus

Authors: *J. M. RICHARD, H. L. FIELDS;
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Abstract: The ventral pallidum (VP) is critical for both seeking and consumption of natural and drug rewards. Although neural activity in the VP encodes the value of both primary rewards and reward-related cues the relationship between VP encoding of reward cues, and reward-seeking actions in response to those cues is relatively unexplored. For this reason, we investigated VP encoding of an instrumental incentive cue, and the relationship of this encoding with cue-induced reward seeking. Rats were trained to perform a discriminative stimulus (DS) task in which lever presses after one auditory cue (the DS) result in presentation of sucrose reward, whereas lever presses after a different auditory cue (the non-predictive stimulus; NS) have no consequences. Following training, rats were implanted with electrode arrays in the rostral or caudal VP, as these subregions may be differentially important for cue-driven behaviors. The majority of neurons in both rostral and caudal VP were excited by the DS, and approximately 2/3 of these excited neurons showed greater excitations to the DS than the NS. Importantly, we found a significant inverse correlation between the firing rate of many of these excited neurons and the animal's latency to make an instrumental response following the DS on a given trial, suggesting these excitations may play a causal role in reward seeking actions following the DS. Approximately half of recorded neurons in caudal VP exhibited DS-generated excitations that were negatively correlated with the latency to make an instrumental response (i.e. higher firing rates predicted shorter latencies to respond). A slightly smaller proportion of rostral VP neurons (~one third) showed this same negative correlation. In rostral VP we also observed a smaller population of neurons (~17%) that were inhibited by the DS, most of which were not inhibited by the NS.

These inhibitions were not observed in caudal VP, and were not significantly related to the latency to respond on a given trial. These results suggest that DS excitations in caudal VP may contribute to controlling the vigor of cue-driven reward seeking responses in the DS task. Ongoing experiments explore the effect of pharmacological agents and the contribution of different inputs to VP for neural encoding and behavioral performance in the DS task, as well as whether these effects differ between rostral and caudal VP.

Disclosures: J.M. Richard: None. **H.L. Fields:** None.

Poster

756. Motivation and Emotions: Reward Circuitry

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Support: Wellcome Trust Investigator Award (096689/Z/11/Z)

FCT Grant SFRH / BD / 51711 / 2011

Title: Evidence of model-based and model-free reinforcement learning in prefrontal cortex and striatal neurons

Authors: *B. MIRANDA[1], N. MALALASEKERA[3], P. DAYAN[2], S. KENNERLEY[4]; [1]Sobell Dept., [2]Gatsby Computat. Neurosci. Unit, Univ. Col. of London, London, United Kingdom; [3]Sobell Dept., Univ. Col. - Inst. of Neurol., London, United Kingdom; [4]Sobell Dept., Univ. Col. of London - Inst. of Neurol., London, United Kingdom

Abstract: Two major competing and cooperating strategies for behavioral control are goal-directed or model-based (MB) reinforcement learning (RL), and habitual or model-free (MF) RL. We studied the interaction of these two RL strategies, and their realization in the activity of striatal and prefrontal neurons. We trained two subjects to perform a two-stage Markov decision task (adapted from Daw et al 2011) that induces a combination of MB and MF behavior. The task starts with a choice between two options. Each of these options more commonly (70%) leads to one of two second-stage states, but less commonly (30%) transitions to the other second-stage state. A second-stage choice between two further options results in one of three different reward outcomes. The outcomes change magnitude independently every 5-9 trials and thus induce exploration. Logistic regression analysis showed that first-stage choice was

predominantly influenced by the interaction between recent rewards and state transitions (of MB importance). A trial-by-trial analysis using different RL algorithms found that in the best fitting model, choices were made by a weighted combination of MF and MB Q-values, with a MB weight of 83% & 71% for the two subjects. Single-neuron activity was recorded from 93 neurons in frontal pole (FP), 51 in anterior cingulate cortex (ACC), 34 in dorsolateral prefrontal and 80 in dorsal striatum (dStr). At feedback, the most common selectivity present in all areas (but strongest in ACC and dStr) was coding of expected outcome. These two regions also encoded recent reward history, but in distinctive ways: dStr activity mostly reflected the temporal difference of successive rewards; ACC neurons coded for a mixture of temporal sum and difference. ACC also encoded information about state transition and [reward x transition] interaction, key elements of MB RL. At the time of first-stage choice, FP cells also encoded the [reward x transition] information about the previous trial. ACC activity predicted which choice would be made, a pattern that first emerged during the feedback epoch of the previous trial. Taking advantage of the Q-values estimated by our models, at feedback both dStr and ACC encoded the updated MF values. However, ACC activity also correlated with the chosen MB value of both the current and future first-stage choices. Our behavioral results support an integrated view of MF and MB learning strategies. The neuronal evidence that ACC tracks reward history, [reward x transition] structure and both MF and MB Q-values suggests a role in arbitrating between MF & MB RL strategies, a function that is crucial for guiding optimal behavior.

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Poster

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Support: NIDA R01DA033396

NIMH F31MH101956

Title: Bimodal role for nucleus accumbens dynorphinergic neurons in aversion and reward

Authors: *R. AL-HASANI[1], J. G. MCCALL[1], J. M. WONG[2], O. M. MABROUK[2], N. A. CROWLEY[3], G. SCHMITZ[1], D. Y. HONG[1], M. J. KRASHES[4], B. B. LOWELL[4], T. L. KASH[3], R. T. KENNEDY[2], M. R. BRUCHAS[1];
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Abstract: The dynorphin/kappa opioid system is implicated in stress and vulnerability to drug abuse. It is thought that stress causes dynorphin release activating kappa-opioid receptors (KOR) within both dopaminergic and serotonergic nuclei and their ventral striatal targets. Consequently, much attention has focused on these systems in the modulation of KOR-mediated responses. Despite our current knowledge of central dynorphinergic cell body populations, a clear description of the axonal projections of these neurons is unknown. To address this we crossed the Cre-dependent tdTomato (Ai9) reporter mouse to a mouse expressing Cre recombinase under the same promoter as dynorphin (Dyn-Cre) so only dynorphinergic cells express tdTomato, allowing complete visualization of dynorphinergic circuitry throughout the brain. We show robust dynorphin expression in cell bodies throughout the brainstem and forebrain. We were also able to use these mice in conjunction with viral retrograde approaches to isolate and identify NAc dynorphinergic projections throughout the brain. Dynorphinergic neurons within the striatum are particularly interesting for the study of stress and drug abuse. Prior studies have shown that KOR agonists inhibit dopamine and serotonin release in the nucleus accumbens (NAc), which regulates aversive behaviors. Therefore, we investigated whether specific modulation of dynorphinergic neuronal firing in the NAc is sufficient to induce aversive behaviors. We virally targeted channelrhodopsin-2 to striatal dynorphinergic neurons and optogenetically activated neuronal populations in both the dorsal and ventral NAc shell. This activation inhibited electrically-evoked EPSCs which was reversed by norBNI application. Activation of dorsal NAc shell induces a place preference and is positively reinforcing in an FR1 operant task paradigm while activation of ventral NAc shell drives conditioned and real-time aversive behavior. This photoactivation of dynorphinergic neurons in the ventral NAc also increased dynorphin release, as measured using microdialysis and mass spectroscopy. Understanding the mechanisms by which the dynorphin/kappa opioid system regulates negative affective behaviors will provide valuable insight into potential treatments for drug abuse and depression. Work supported by NIDA R01DA033396 (M.R.B, R.A), NIMH F31MH101956 (J.G.M). Authors declare no conflict of interest.

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Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: NIDA 1R21DA034929-01A1

Title: Dissecting nociceptin receptor modulation of reward

Authors: *S. SPANGLER[1], E. E. PETERSON[1], R. AL-HASANI[1], W. PLANER[1], J. MCCALL[1], G. D. STUBER[2], T. KASH[3], T. JHOU[4], M. R. BRUCHAS[1];
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Abstract: Nociceptin/Orphanin FQ Opioid Receptor (NOPR) and its endogenous ligand, nociceptin (NOFQ) have been shown to affect the rewarding properties of drugs of abuse. Both NOPR and N/OFQ are highly expressed in the Ventral Tegmental Area (VTA), a widely regarded as a critical anatomical region for both drug and natural reward. The circuitry of the NOPR/NOFQ receptor system in the context of reward, however, remains unknown. The aim of this study was to dissect the role of NOPR and NOFQ in reward modulation. First, we pharmacologically targeted NOPR receptors with potent and selective NOPR agonist, SCH 221510. Consistent with prior studies on NOPR agonism, we demonstrated that SCH221510 significantly attenuates drug-seeking behavior in a cocaine conditioned place preference (CPP) model. Then, to determine whether the effect of SCH221510 was specific to NOPR expressed in dopaminergic VTA (DA-VTA) neurons, we targeted a cre-dependent NOPR virus into the VTA of NOPR knockout crossed with Tyrosine hydroxylase-Cre mice. Our preliminary data suggests activation of NOPR specifically within the dopaminergic neurons of the VTA is sufficient to attenuate drug-seeking behavior in a CPP model. To determine the source of endogenous nociceptin to the VTA that modulates reward behavior, we generated a novel Cre mouse line, Noci-IRES-Cre, in which the promoter for the NOFQ gene drives Cre recombinase expression. We then created a reporter mouse by crossing a Noci-Cre mouse with a Cre-dependent tdTomato (Ai9) reporter mouse. In this reporter mouse, tdTomato is expressed in NOFQ positive neurons and allows us to examine both cell bodies and projection sites. We show NOFQ is expressed in the expected cell bodies of brain loci involved in reward and affective behavior, including the Rostromedial Tegmental nucleus (RMTg) and the VTA, which inhibit DA-VTA neurons, and also the Central Amygdala (CeA). We optogenetically targeted these nociceptin containing cells

and evaluated their effects on reward and aversion behavior. These data provide the groundwork for NOPR containing neural circuits that regulate reward and aversive-like behavior.

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Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: ERC

Title: Dopaminergic and noradrenergic systems contribution to motivation: Pharmacological study in rhesus macaques

Authors: *C. I. JAHN[1,2], N. BORDERIES[1,2], C. VARAZZANI[1,2], S. BOURET[1,2];
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Abstract: Motivation refers to what drives behavior. Behavior is generally driven by the incentive effect of reward, and we tend to produce more effort when more reward is at stake. However, there are situations in which the amount of energy invested in the action does not flow directly from the outcome value, for instance when the effort is imposed and there is no obvious reward. This suggests that a specific neural system can underlie effort production independently of the expected reward. Several lines of research have described the major contribution of the dopaminergic system to incentive motivation, but its role in effort production remains debated. The central noradrenergic system, given its implication in autonomic and behavioral mobilization to face potential challenges, might be critical for reward-independent effort production. We are testing these ideas using a pharmacological approach in monkeys. We trained 3 monkeys in a task where the amount of reward they get is proportional to the amount of physical effort they produce. Each trial starts with a cue indicating the range of rewards at stake (low, medium, high). The fraction of the stake that they obtain is directly proportional to the force produced. Thus, monkeys must find a trade-off between the amount of reward that they want and the cost associated with the corresponding effort. In a pilot version of these experiments with 2 monkeys, we used atomoxetine (1 mg/kg) and L-DOPA (20 mg/kg), to

increase the function of noradrenaline (NA) and dopamine (DA), respectively. Atomoxetine reliably enhanced the amount of force produced and the acceptance rate. The effects of L-DOPA were less robust. We used a computational model to describe the cost of effort and the incentive effect of expected reward on the force produced. Preliminary analysis indicate that L-DOPA increases the parameter that mediates the incentive effect of reward on exerted force, whereas atomoxetine decreases the parameter scaling the cost of effort. These preliminary data are in line with the hypothesis of a complementary action of DA and NA on reward dependent and reward independent.

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Poster

756. Motivation and Emotions: Reward Circuitry

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Topic: F.03. Motivation and Emotion

Support: ERC

Title: Differential integration of reward value and effort cost by noradrenaline and dopamine neurons: A direct comparison in behaving monkeys

Authors: *C. VARAZZANI, A. SAN-GALLI, S. BOURET;
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Abstract: Neuromodulation is essential for various aspects of behavior, but to what extent each neuromodulator contributes to drive and motivate energy expenditure remains unclear. The role of dopamine (DA) is probably the most clearly defined computationally, with convergent studies confirming its role in mediating the influence of reward on subsequent choices. Conversely, the role of noradrenaline (NA) is less clear: although robust evidence shows that NA plays a crucial role in arousing behavior and it is a main target of drugs for motivational deficits, a precise assessment of the NA contribution to motivation is still lacking. In this study, 62 NA neurons from locus coeruleus (LC) and 80 DA neurons from substantia nigra pars compacta (SNc) were recorded and compared in three rhesus macaques. In each trial, monkeys must produce a given effort (3 levels of force) in order to obtain a given reward (3 reward sizes). Abandon rates rise as reward decreases and effort increases, indicating that the reward value is discounted by the amount of physical effort required to obtain it. Both NA and DA neurons show a significant

increase ($p<.01$) in firing rates 100-400 ms after cue onset (DA: 40%; NA: 25%) and -100-400 ms around effort onset (DA: 35%; NA: 62%). Population-level analyses reveal that while the expected reward positively modulates the cue-evoked activity in both systems (DA: $p<.01$, NA: $p<.01$), the anticipated effort cost negatively affects only the DA neurons (DA: $p<.05$). Furthermore, DA cue-activity is predictive of both the subsequent trial acceptance (DA: $p<.05$) and the amount of exerted force in the upcoming action (DA: $p<.01$). During the action itself, we found that only the response magnitude of NA neurons scale with the level of imposed effort (NA: $p<0.001$) and this activity correlates positively with the amount of exerted force (NA: $r=.17$, $p<.01$) and with pupil dilation (NA: $r=.13$, $p<.01$), while DA neurons do not. We conclude that NA and DA neurons play a complementary role in the integration of the expected value and the energetic demands of upcoming actions. On the one hand, DA cells encode the action value by increasing proportionally to the expected reward and decreasing with the anticipated effort cost. On the other hand, NA cells encode the energetic demands by boosting behavior proportionally to the required amount of effort.

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Poster

757. Human Reward

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Title: The neural basis of familial obesity risk: fMRI responses to food words in lean adolescents with obese/overweight mothers

Authors: *S. CARNELL[1], L. BENSON[1], V. K.-Y. CHANG[2], Z. WANG[2], A. GELIEBTER[2], B. S. PETERSON[2];
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Abstract: Objective: The offspring of obese/overweight parents are at increased risk of becoming obese. Identifying the biobehavioral factors that promote weight gain in this group - as well as factors that might protect them from excessive weight gain - could help guide interventions. Our primary goal was to compare neural and behavioral responses to food cues, as

well as appetitive trait scores, ad libitum food intake, and physical activity, in lean adolescents at high vs. low familial risk of obesity. Methods: Twenty-six lean adolescents, and 10 obese/overweight adolescents participated. Of the lean adolescents, 16 had obese/overweight biological mothers (lean high-risk [lean-HR]), and 10 had lean biological mothers (lean low-risk [lean-LR]). Following a ≈5h fast, all adolescents participated in an fMRI scan during which they viewed two-word phrases representing high energy-density (ED) and low-ED foods, and rated their desire to eat each food. They were then given a multi-item ad libitum meal to measure intake. Adolescents completed the Power of Food Scale and mother completed questions about adolescents' physical activity. Results: In response to high-ED (vs. low-ED) foods, both lean-HR and obese/overweight adolescents showed less activation in the cingulate cortex (anterior, middle, posterior), when compared with lean-LR adolescents. There were also group differences in several frontal structures such that lean-HR and obese/overweight adolescents showed relatively decreased responses to high-ED foods. Lean-HR adolescents reported greater desire to eat the high-ED foods than both the lean-LR and obese/overweight groups ($p<.05$). The lean-HR group also had higher Power of Food scores ($p<.05$) and participated in more after-school/weekend physical activity ($p<.05$) than the other groups. Intake was highest for the obese/overweight group, followed by the lean-HR, then the lean-LR group ($p<.01$). Conclusions: Like obese/overweight adolescents, lean-HR adolescents showed relatively decreased high-ED food cue responses in circuits involved in self-regulation and control, when compared to lean-LR adolescents. Lean-HR adolescents additionally showed heightened appetitive responses to high-ED food cues and increased intake compared to other lean teens. However, they also reported greater physical activity participation compared to both lean-LR and obese/overweight teens. The altered brain responses and obesogenic eating styles we observed might increase risk of future weight gain in adolescents with obese mothers - but this risk might be offset by protective behaviors such as physical activity.

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Poster

757. Human Reward

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Support: JSPS KAKENHI No. 25350993

Title: Synchronized brain responses during passive viewing of a humorous movie

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Abstract: Sense of humor is highly specific to humans and, therefore, considered a feature that distinguishes humans from other primates. The neuroanatomical substrates of humor sensation have been investigated by performing functional and structural imaging and in patients with brain damage. These studies showed that the ability to perceive humor is subserved by neural activity in social and reward circuits involving the prefrontal and temporal cortices, ventral striatum, and amygdala. In the present study, we investigated how synchronized brain activity during viewing of comedy movies relates with subjective feelings of humor by using functional magnetic resonance imaging (fMRI) and inter-subject correlation (ISC) analysis. Thirty-five healthy volunteers took part in the experiments (male/female: 21/14, mean age: 21 years). Participants viewed an 8-m video clip of the movie *Mr. Bean* with no audible contents presented. The movie depicted mostly humorous scenes, was understandable for all participants, and had not been previously viewed by any of the participants. fMRI was performed for 14 participants during passive viewing of the video, and no overt responses were required. Whole brain scanning was conducted using 3T MRI with a 2.5-s repetition time. The remaining 21 participants rated subjective feelings of humor every 7 s by using a 4-button response box (from 1: least humorous to 4: most humorous) during movie viewing. The imaging data were pre-processed using SPM8 and synchronized brain activity was analyzed using the ISC toolbox. In the ISC analysis, the mean of the voxel-wise correlation coefficients across all possible subject pairs was computed. The obtained ISC maps reflected the degree of similarity in brain responses across participants in 3 frequency sub-bands (0-0.05 Hz, 0.05-0.1 Hz, and 0.1-0.2 Hz). To investigate the dynamic nature of brain activity during naturalistic viewing, 90-s moving averages of the ISC map with 23-s step length were computed. ISC measures of 19 time-windows in 64 brain regions were extracted and correlated with the mean subjective rating obtained during the movie viewing. The main finding was that ISCs in the right amygdala and anterior part of fusiform gyrus significantly ($p = 0.01$) and positively ($r = 0.58$-0.66) correlated with the rating in 2 frequency sub-bands. These results indicate that the greater the synchronization in neural activity, the greater the subjective feelings of humor. In conclusion, neural synchrony in the amygdala and fusiform gyrus, which are involved in processing human faces and bodies, may play a critical role in the subjective sense of humor.

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Poster

757. Human Reward

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Topic: F.03. Motivation and Emotion

Support: The University of Tulsa Student Research Grant Awarded to Jennifer DelVentura

Title: Prepulse inhibition of startle is reduced by highly palatable food stimuli in men relative to women

Authors: *S. PALIT, J. DELVENTURA, B. KUHN, M. PAYNE, L. FITTER, D. SIMON, J. RHUDY;
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Abstract: Obesity, measured as a body mass index greater than 30, has become more prevalent in recent years. Moreover, men tend to have a greater propensity for becoming obese or overweight compared to women. Therefore, it is important to investigate potential mechanisms underlying sex differences in obesity. One such mechanism may be differences in dopaminergic activation in response to food cues (e.g., anticipation of food reward). Prepulse inhibition (PPI) of the startle reflex (presentation of a non-startling prepulse immediately preceding an acoustic startle probe that results in a reduction of the startle magnitude) is related to dopamine activity. Specifically, increases in dopamine levels are associated with reduced PPI (less startle inhibition). Thus, individual differences in dopamine activity can be inferred from differences in PPI. Sex-related disparities in obesity could be stemming from differences in dopaminergic activation in men in the context of highly palatable foods (e.g, increased craving). The current study investigated PPI modulation via affective states by using a paradigm whereby participants viewed emotionally-charged pictures varying in content (erotica, high fat/sugar foods, low fat/sugar foods, neutral, and attack). Startle probes were presented over headphones with and without prepulses during these pictures and startle magnitudes were recorded. In addition, participants made pleasantness, unpleasantness, and arousal ratings of the pictures. Participants were 67 healthy individuals (43 female). Results indicate that men and women differed on modulation of PPI such that men evidenced less PPI during high fat/sugar food pictures compared to women ($p = .01$). Consistent with previous research, men rated erotic pictures as more pleasant than women ($p < .001$), but women rated erotica and the high fat/sugar food pictures as more unpleasant than men ($p < .001$). Sex differences in arousal ratings were found for attack and erotic pictures, such that men rated both of these picture contents as more arousing than women ($p < .001$). These findings suggest that there are sex differences in modulation of PPI, but only by high/fat sugar food cues. This implicates dopaminergic reactivity to food as a possible mechanism contributing to sex differences in obesity.

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Poster

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Topic: F.03. Motivation and Emotion

Support: KAKENHI (24240061)

Title: The neuroanatomical basis of general self-efficacy

Authors: ***A. SUGIURA**[1,2], R. AOKI[2,3,4], Y. YOMOGIDA[3], M. MATSUMOTO[3], K. MURAYAMA[5], K. IZUMA[2,3,4], T. HAJI[6], A. SAITO[1], T. HASEGAWA[1], K. MATSUMOTO[3]; [1]Dept. of Life Sciences, GSAS, Univ. of Tokyo, Tokyo, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Brain Sci. Institute, Tamagawa Univ., Tokyo, Japan; [4]Caltech, Pasadena, CA; [5]Univ. of Reading, Berkshire, United Kingdom; [6]Brain Activity Imaging Center, ATR-Promotions, Kyoto, Japan

Abstract: General self-efficacy is the strength of belief one has in one's own overall ability to successfully perform tasks, and general self-efficacy scores predict performance over time. We investigated the neural basis of individual differences in general self-efficacy by examining regional gray matter volume using voxel-based morphometry (VBM) analysis. This approach was chosen because general self-efficacy is a personality characteristic that is difficult to manipulate rapidly during a single experiment. Brain anatomy was analyzed using magnetic resonance images obtained from 64 healthy right handed participants (mean age = 20.1 ± 1.4 years, 29 males) who had completed Sherer's general self-efficacy scale. After controlling for the effects of age, sex, and total gray matter volume of each subject, results showed that regional gray-matter volume in the precuneus was significantly and positively correlated with general self-efficacy scores ($z = 4.13$, $P = 0.001$, cluster-level FWE corrected), and that the volume of a cluster in the left medial prefrontal cortex (mPFC), which is functionally and anatomically connected to the precuneus, followed the same trend ($z = 4.87$, $P = 0.059$; cluster-level FWE corrected). No similar association was seen between gray-matter volume and scores on other questionnaires(Self-esteem, BIS/BAS, Achievement motivation). Several separate studies show that recall of autobiographical information is associated with gray-matter volume in the precuneus (Frenton et al., 2013) and with general self-efficacy (Hallford et al., 2013), and that

depression is linked to both lower gray-matter volume in the mPFC (Frodl et al., 2008) and lower general self-efficacy (Chang et al., 2011). Here, our direct comparison is consistent with these results, and indicates that the gray matter volume in the precuneus and mPFC is related to individual differences in general self-efficacy.

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Poster

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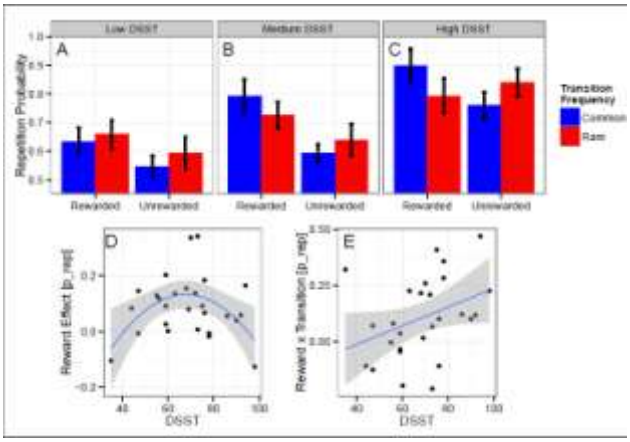
DFG FOR1617 grant ZI1119/4-1

Title: Smart goals, slow habits? Individual differences in processing speed and working memory capacity moderate the balance between habitual and goal-directed choice behaviour

Authors: *Q. J. HUYS[1], D. J. SCHAD[2], E. JUENGER[3], M. SEBOLD[2], M. GARBUSOW[2], N. BERNHARDT[3], A. JAVADI[4], U. ZIMMERMANN[3], M. SMOLKA[3], A. HEINZ[2], M. RAPP[2,5]; [1]Translational Neuromodeling Unit / UZH & ETHZ, Zurich, Switzerland; [2]Dept. of Psychiatry and Psychotherapy, Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Dept. of Psychiatry and Psychotherapy, Technische Univ. Dresden, Dresden, Germany; [4]Inst. of Behavioural Neurosci., Univ. Col. London, London, United Kingdom; [5]Ctr. of Excellence in Cognitive Sci., Univ. Potsdam, Potsdam, Germany

Abstract: Background: Choice behaviour is shaped by cognitively demanding goal-directed and by more automatic habitual processes. External cognitive load manipulations alter the balance of these systems. However, it is unclear how individual differences in specific cognitive abilities contribute to the arbitration between habitual and goal-directed decision-making. Method: 29 adults performed a two-step decision task explicitly designed to capture the two systems' computational characteristics. We also collected measure of fluid and crystalline intelligence. Results: There was an inverted U-shape relationship between processing speed and habitual choice together with a linear relationship between processing speed and goal-directed behaviour. Working memory capacity impacted on this balance only amongst those subjects with high processing speed. Conclusion: Different aspects of intelligence have specific contributions to complex human decision-making. Individual differences in such cognitive abilities moderate the balance between habitual and goal-directed choice behaviour.



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Title: Dopaminergic malfunction: The link between impulsivity and reinforcement learning?

Authors: *S. NEBE[1], N. B. KROEMER[1], D. J. SCHAD[2], M. SEBOLD[2], F. SCHLAGENHAUF[2], A. HEINZ[2], Q. J. M. HUYS[3], M. A. RAPP[4], M. N. SMOLKA[1]; [1]Section of Systems Neurosci., Technische Univ. Dresden, Dresden, Germany; [2]Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Translational Neuromodeling Unit, ETH Zürich and Univ. of Zürich, Zürich, Switzerland; [4]Social and Preventive Med., Univ. of Potsdam, Potsdam, Germany

Abstract: Increased impulsivity is a well-established risk factor for the development of addictive behavior. Dopamine neurotransmission may be one neurobiological mechanism linking these two. The reward prediction error (RPE) signal is closely linked to dopaminergic function in ventral tegmental area (VTA), striatum, and ventromedial prefrontal cortex (vmPFC). RPE acts as a teaching signal by transcoding unexpectedly rewarded or punished behavior, thereby facilitating model-free reinforcement learning on the basis of reward history and driving habitual behavior. A second, model-based, goal-directed system relies on an internal model of transition probabilities between states and their associated reward value. Here, we examined the relationship between two facets of impulsivity and model-based and model-free learning. Impulsive choice as aspect of impulsivity was operationalized as delay and probability discounting to assess the preference for immediate rewards and risk taking, respectively. Model-free and model-based learning were measured in a two-stage Markov decision task during fMRI. 47 healthy male 18-year-old participants performed all tasks as part of an ongoing study on learning and alcohol abuse. First-level analyses of fMRI data were set up according to Daw et al. (2011) comprising model-free RPE and a difference regressor of model-based and model-free RPE. The behavioral measures of impulsivity were the discount rates kDD (delay discounting rate) and kPD (probability discounting rate), for which we assumed a hyperbolic discounting model. We estimated both ks by sampling their full posterior distribution and updating it according to a Bayesian fashion on a trial-by-trial basis. ks were log-transformed and included in second-level analyses. For whole-brain and ROI analyses, we used a threshold of pFWE<.05 (peak). We found a negative association of impulsive choice in delay and probability discounting with model-based correlates of BOLD signal in striatum and VTA. However, there were no significant correlations with BOLD correlates of model-free learning. This link between model-based RPE signals in the dopaminergic system and impulsivity raises questions about whether aspects of impulsivity might be mediated via dopaminergic influences on goal-directed decision-making. Thus, since model-based signals supposedly update the model-free learning system, this finding suggests that impulsive individuals could profit less from receiving unexpected reward feedback which could promote the development of addictive behavior as well as its maintenance due to decreased effectiveness of cognitive-behavioral therapies.

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Poster

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DFG Grant SM80/7-1

DFG Grant SFB 940/1

Title: By force of habit: Hyper- and hyporeactivity to reward in orbitofrontal cortex in overweight

Authors: ***N. B. KROEMER**, S. POOSEH, F. WUTTIG, M. N. SMOLKA;
Psychiatry and Psychotherapy, TU Dresden, Dresden, Germany

Abstract: Obesity is one of the main preventable causes of death in the developed countries today. Escalating food intake has been commonly associated with a breakdown of goal-directed control of eating behavior. However, theories disagree if this runaway process is directed by hyper- or hyporeactivity to food reward in overweight individuals and if it generalizes to secondary reinforcers. We hypothesized that the balance between habitual and goal-directed behavior in instrumental motivation for monetary reward would be associated with differences in reward processing that can be linked to diverging overweight phenotypes. Using fMRI, we investigated 100 participants (52 female; mean age=36.8 years; mean BMI=24.4 kgm-2) employing an instrumental motivation task. In this paradigm, a cue indicated reward levels before the onset of a 3-s instrumental response phase. We assumed that the action (i.e., button presses) is a weighted sum of habitual and goal-directed (cue sensitive) behaviors and estimated a parameter (ω) to capture goal-directedness. As a priori ROIs, we defined the ventral and dorsal

striatum, and the lateral and medial orbitofrontal cortex. Based on extracted signal, we used multilevel analysis to estimate single-trial betas for every reward level to fit subjective value functions. In all ROIs, habitual behavior was associated with attenuated slopes of subjective value for cue responses, but elevated brain responses at onset of the response phase. Whereas BMI was not correlated with ω ($r = .07$), it was correlated with the overall instrumental-response rate ($r = .24$; $p = .017$). Critically, BMI interacted with ω for cue responses in both lateral and medial orbitofrontal cortex, but not in striatum. The interaction was characterized by elevated cue-responses in overweight+habitual individuals, specifically to the low and medium reward level, and attenuated cue-responses to the highest reward level in overweight+goal-directed compared to normal-weight individuals. To summarize, our findings suggest that both sets of theories of the runaway process involved in the etiology of overweight/obesity might be correct, applying, in fact, to different endophenotypes. Goal-directedness in response to reward is thought to be an indication of enhanced dopaminergic function which is in line with observed differences in value tracking. However, the interaction with BMI was constrained to the orbitofrontal cortex which is involved in long-term credit assignment of cues and the transfer of subjective value to action. Consequently, habitual vs. goal-directed behavior to reinforcement in general might be a promising candidate in tailoring intervention strategies.

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Poster

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Title: Trading goals and habits: the role of dorsolateral PFC structure supporting working memory

Authors: *D. J. SCHAD[1], M. GARBUSOW[1], M. SEBOLD[1], E. FRIEDEL[1], C. HÄGELE[1], S. NEBE[3], N. B. KROEMER[3], H. WALTER[1], J. GALLINAT[2], M. N. SMOLKA[3], A. HEINZ[1], M. A. RAPP[1,4], T. WÜSTENBERG[1], Q. J. M. HUYS[5];
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Abstract: Distinct neural systems for habitual (model-free) versus goal-directed (model-based) choices are key to complex human decision-making. Imaging, electrophysiological interference and pharmacological results point to the lateral PFC as an important mediator of the balance between the two systems. The lateral PFC is also known to be involved in working memory and other neural processes that relate to individual variation in this trade-off. We therefore asked whether structural aspects of the lateral PFC might relate to the individual variation in the trade-off between model-based and model-free decision-making. 130 subjects underwent structural and 91 subjects functional MRI while performing a Markov-decision task. We performed voxel-based morphometry and model-based computational fMRI analysis. Subjects with stronger model-based (goal-directed) components in their behavior had higher grey matter density in the dorsolateral prefrontal cortex (dlPFC; p_FWE whole brain = .05, t = 4.71). At this site, grey matter density was also associated with a larger working memory capacity (p_FWE ROI = .04, t = 2.52), and fMRI BOLD showed a model-based reward prediction error signal (p_FWE ROI = .03, t = 2.73). These results suggest that structural variation in the human brain may contribute to individual variation in decision-making. Higher grey matter density in the dlPFC may support working memory processes facilitating model-based control of choice behavior.

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Poster

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Topic: F.03. Motivation and Emotion

Support: LIU sabbatical grant to JCN

Title: Modulation of goal-directed versus habitual behavior by seizure- induced stressors

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Psychology, Long Island Univ., GREENVALE, NY

Abstract: Patients with intellectual disabilities and epilepsy are often managed with behavior modification programs that may work poorly. One reason is lack of attention to the effects of repeated stressors which predispose the patient to act habitually rather than goal oriented, i.e., a lack of discrimination accuracy ensues following a chronic acute stressor such as a generalized seizure. The present paper will present human and non-human data collected using an analog operant behavior analysis system. Several models of seizure propagation in rat (kainic acid, pilocarpine, flurothyl-induced seizures) show seizures predispose the organism to ignore discriminative stimuli associated with reward/non-reward, and to increase the rate of operant behavior that had been extinguished. Similar results are obtained in patients with intellectual disabilities and intractable epilepsy. Using extinction as a therapeutic intervention for maladaptive repetitive behavior, which is a common clinical tool, works very poorly to change this particular clinical behavior pattern in this kind of patient, because the seizure, in addition to evoking short term amnesia, also causes a physiological stress reaction, elevated glucocorticoids, and a resulting rise in unreinforced responding and a loss of stimulus control. In an attempt to preempt this long term pattern, we provided early enrichment with contingent reinforcement to rats that either had or didn't have seizures early in life. This is similar to early intervention with Applied Behavior Analysis used with autistic and epileptic children. In animals, this enrichment led to improved discrimination behavior and less errors, except in the presence of stressors. These results will be related to earlier work in our lab, which showed changes in D2 receptor density in the striatum; elevations following early intervention with cue-based reinforcement; and, lower levels following seizures.

Disclosures: J.C. Neill: None.

Poster

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Aalto University

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The National Graduate School of Clinical Investigation

University of Turku

Åbo Akademi

Title: Weight loss driven recovery of μ-opioid receptor availability is associated with lowered trait anxiety in the morbidly obese

Authors: H. K. KARLSSON[1], J. J. TUULARI[1], L. TUOMINEN[1], J. HIRVONEN[2], P. SALMINEN[3], P. NUUTILA[1], *L. NUMMENMAA[4];
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Abstract: Background: Previous positron emission tomography (PET) studies suggest that weight loss normalizes initially lowered μ-opioid receptor (MOR) availability in the morbidly obese but does not affect dopamine D2 receptor (D2R) availability in the human brain. Endogenous opioid system is involved in neuromodulation of anxiety, but it remains unknown whether altered MOR availability is related to experienced anxiety in obesity. We hypothesized that i) MOR availability is negatively associated with anxiety and ii) recovery of MOR availability is associated with reduced anxiety among morbidly obese. Methods: We recruited 21 morbidly obese women (mean BMI 41, mean age 42), eligible for bariatric surgery, and measured their brain MOR availability using PET with [11C]carfentanil and D2R availability with [11C]raclopride before and six months after the operation. Subjects completed trait anxiety inventory (STAI) on both visits. 14 non-obese healthy women (mean BMI 23, mean age 45) formed the control group. ROIs were delineated in the ventral striatum, dorsal caudate, putamen, insula, amygdala, thalamus, orbitofrontal cortex and anterior, middle and posterior cingulate cortices. Binding potentials (BPND) were extracted from both pre- and postoperative scans. Association between receptor availability and anxiety was assessed by computing Pearson correlations between initial BPNDs as well as BPND change scores. Results: Preoperatively obese subjects had significantly lower MOR availability than controls in all ROIs as well as higher STAI scores ($p = 0.035$). MOR availability and STAI scores were negatively associated in all ROIs except for ventral striatum and amygdala ($rs < -0.33$, $ps < 0.05$). Postoperatively, obese had on average 31 % higher MOR availability compared to preoperative state after mean weight loss of 25 kg, and there was no difference in STAI scores between obese and control groups ($p = 0.36$). Increases in MOR availability were associated with decreases in STAI scores in all ROIs

(rs < -0.46, ps < 0.05). Weight loss did not influence D2R availability in any brain region and no significant associations between STAI scores were found. Conclusions: Altered MOR system functioning is associated with anxiety among obese, yet this effect is abolished after weight loss. Thus, obesity might be partly maintained by inability to control emotional arousal states, such as anxiety. Understanding the opioidergic contribution to overeating and anxiety is thus critical for developing new psychological and pharmacological treatments for obesity.

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Poster

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Program#/Poster#: 757.11/VV23

Topic: F.03. Motivation and Emotion

Support: ZonMW TOP grant 91210041

Title: Oxytocin administration modulates striatal and prefrontal responses to reward

Authors: *L. NAWIJN[1], M. VAN ZUIDEN[1], S. B. J. KOCH[1], J. L. FRIJLING[1], D. J. VELTMAN[2], M. OLFF[1,3];
[1]Psychiatry, Academic Med. Ctr., Amsterdam, Netherlands; [2]Psychiatry, VU Univ. Med. Ctr., Amsterdam, Netherlands; [3]Arq Psychotrauma Expert Group, Diemen, Netherlands

Abstract: Background Dysfunctional reward functioning is present in several stress-related, mood- and anxiety disorders, including posttraumatic stress disorder, depression, social anxiety disorder and obsessive compulsive disorder. In humans, intranasal administration of the neuropeptide oxytocin (OT) has been found to influence neural responses to social reward (e.g. Groppe, 2013; Scheele, 2013). However, reward deficits in psychiatric disorders are not limited to social stimuli but extend to non-social stimuli such as money rewards (e.g. Zhang, 2013). The effects of OT administration on responses to monetary reward have not been investigated yet. Therefore, we investigated the effects of intranasal OT on neural processing of monetary reward and loss in healthy participants. **Methods** In a randomized double-blind placebo-controlled cross-over fMRI study, we investigated neural responses to a monetary incentive delay task (MID, Knutson, 2001) in 19 healthy males. Participants were scanned after intranasal OT (40IU) and placebo administration, in counterbalanced order. Small volume corrected analyses were

performed for prefrontal and striatal regions of interest known to be involved in reward processing. **Results** Intranasal OT administration was associated with increased left caudate and right anterior cingulate cortex reactivity during anticipation and presentation of monetary reward compared to placebo administration. During anticipation and presentation of monetary loss, OT administration did not alter striatal responses but increased bilateral OFC responses. **Conclusion** These results are the first to indicate that OT modulates neural responses in the reward-pathway in response to monetary reward and loss. Our findings extend previous findings of OT effects on social reward, and suggest that OT affects salience processing of not only social but also monetary reward. Oxytocin therefore seems an interesting candidate for improvement of social and non-social reward functioning in stress-related, mood- and anxiety disorders.

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Poster

757. Human Reward

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Topic: F.03. Motivation and Emotion

Support: MRC Doctoral Training Account studentship to P. L. L

Title: Prosocial learning: Vicarious reinforcement when choosing for others

Authors: *P. L. LOCKWOOD[1], M. A. J. APPS[3], J. P. ROISER[2], E. VIDING[1];
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Abstract: Our decisions are guided by learning from the outcomes of previous choices. In reinforcement learning theory prediction errors - the difference between a predicted and actual outcome of a choice - act as a neural signal to drive learning. The neural and behavioural mechanisms of learning from reinforcement delivered to oneself are increasingly well understood. However, less is known about how we process rewards delivered to other people. In this study we examine reinforcement learning when making choices for oneself, or when making them on behalf of another. To be motivated to make beneficial decisions for another person (i.e. behave prosocially), it may be critical to represent and vicariously process rewards that others receive following our choices.Participants performed a simple reinforcement learning based task

in which they were required to learn the probability that each of two stimuli would be rewarded. One stimulus was associated with a high probability (75%) of receiving a reward and the other with a low probability (25%). Participants performed this task in three different conditions. They were instructed that either all outcomes would be received by themselves (self reinforcement condition), all outcomes would be received by another participant (confederate, vicarious reinforcement condition), or all outcomes would be received by no one (no reinforcement, control condition). Using a simple reinforcement learning based computational model, we examined the learning rates in each condition. We found a higher learning rate when learning for self compared to no one, and for the other compared to no one conditions, but crucially, there was no difference between the learning rate for self and other conditions. Participants also reported feeling more positive when obtaining rewards for themselves and others compared to no one, providing additional support that participants experienced obtaining rewards for others as vicariously rewarding. These findings suggest that rewards are vicariously experienced when making choices for others. Moreover, the same computational mechanisms appear to drive self and vicarious reinforcement learning. These findings pave the way for examining the neural mechanisms of vicarious reinforcement learning and for studying social behaviour in psychiatric disorders that are characterised by low prosocial motivation, such as psychopathy and autism.

Disclosures: **P.L. Lockwood:** None. **M.A.J. Apps:** None. **J.P. Roiser:** None. **E. Viding:** None.

Poster

757. Human Reward

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

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Topic: F.03. Motivation and Emotion

Support: Singapore TCR Flagship Programme

National Research Foundation, Singapore

Title: How reward information reaches VTA depends on task context: A DCM study

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Abstract: Motivation translates goals into action, and motivation to obtain reward is thought to depend on mesolimbic and mesocortical dopamine systems, namely projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) and to the dorsolateral prefrontal cortex (dlPFC), respectively. How do the dynamic interactions between these regions relate to reward-motivated behavior? How does information about potential reward propagate through the brain? To study the influence of reward motivation on these regions of interest (ROIs) and their interactions, we used dynamic causal modeling (DCM) to analyze functional magnetic resonance imaging (fMRI) data collected as subjects anticipated and prepared for the opportunity to gain reward. A previous DCM fMRI study of the same task suggested that of ROIs, the dlPFC was the sole entry point for reward information as subjects anticipated and prepared for the opportunity to obtain monetary reward for themselves[1]. Anticipated reward thus influenced VTA and NAcc activation via its more direct effect on the dlPFC. Crucially, these data were collected during a modified monetary incentive delay (MID) task in which subjects had opportunities to gain or lose monetary rewards either for themselves or for charity[2,3]. In the present study, we collected fMRI data as subjects performed the same MID task, but with the following key differences: all gains and losses were for the participant, and the magnitude of these potential gains and losses were cued by pictures of monetary bills rather than task-specific arbitrary symbols. DCM analysis revealed that, unlike in the previous study, all three ROIs - dLPFC, VTA, NAcc - were entry points for reward information, which also modulated interactions between the ROIs. Taken together, these findings suggest that although information about reward can reach the VTA via multiple routes, the dlPFC is the exclusive entry point to the circuit when current context determines the value of reward cues. Footnotes [1] Ballard IC, Murty VP, Carter RM, MacInnes JJ, Huettel SA, Adcock RA. (2011). *J Neurosci* 31(28):10340-10346. [2] Carter RM, MacInnes JJ, Huettel SA, Adcock RA. (2009). *Front Behav Neurosci* 3:21. [3] Knutson B, Adams CM, Fong GW, Hommer D. (2001). *J Neurosci* 21:RC159(1-5).

Disclosures: **M.T. Vu:** None. **E.J. Sumner:** None. **I.C. Ballard:** None. **V.P. Murty:** None. **S.A. Chong:** None. **M. Subramaniam:** None. **M.S. Kraus:** None. **J.S. Poh:** None. **K. Dorairaj:** None. **S.N. Yaakub:** None. **J.Y.J. Thong:** None. **J. Lee:** None. **M.W. Chee:** None. **R.S.E. Keefe:** None. **R.A. Adcock:** None.

Poster

757. Human Reward

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Topic: F.03. Motivation and Emotion

Support: NIMH Grant K01MH096175-01

Oklahoma Tobacco Research Center

NIMH IRP

NIDDK IRP

Title: The relationship between food cognitive restraint and brain activity while anticipating and receiving food rewards

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Abstract: Although self-reported measures of cognitive restraint on eating reflect intentional restriction of eating to maintain or lose weight, they are poor predictors of caloric intake and body weight. Food cognitive restraint (FCR) likely reflects a complex interaction of neural responses both to food cues and the experience of eating. Although FCR is expressed behaviorally in the presence of immediately available food rewards, most neuroimaging studies have examined its relationship to activity simply in response to food pictures. It is therefore unclear how FCR is manifested in brain activity in a more behaviorally relevant context of anticipating and receiving food rewards. We asked 24 healthy adults to complete a self-report measure of FCR and then undergo an fMRI task measuring responses to anticipated and received liquid rewards. In the scanner, subjects saw either the word “SWEET” or “NEUTRAL” for 5 s, followed immediately by the word “TASTE” for 5 s, during which time they received 0.4 ml of either a pleasant sweet tastant or a tasteless solution. MRI data were collected on a GE 3 Tesla scanner with 16-channel head coil. Group analyses of the fMRI data indicated that FCR was positively correlated with caudate and posterior insula activity upon receiving liquid rewards. In contrast, FCR was negatively correlated with dorsal mid-insula activity in response to cues preceding those liquid rewards. In other words, greater FCR is reported in individuals who experience stronger caudate and posterior insula activity while consuming food rewards. In those same individuals, however, greater FCR may attenuate insula activation to cues predicting rewarding foods. FCR’s relationship to consummatory and anticipatory brain activity is even more pronounced when the magnitude of response to food stimuli are expressed relative to the magnitude of response to food cues. Within the insula, putamen, caudate, and amygdala, strong positive correlations were observed between FCR and the difference in activity upon receiving

liquid rewards relative to activity to cues predicting those rewards. No associations were observed between FCR and prefrontal regions traditionally associated with successful emotion regulation and cognitive control of behavior, a finding that agrees with evidence that high FCR does not result in reduced caloric intake or weight loss. In summary, within the context of immediately available food rewards, individuals who utilize high cognitive restraint on eating experience greater activity associated with food consumption, and attenuated food anticipation activity, within limbic and paralimbic structures.

Disclosures: **K. Burrows:** None. **J.E. Ingeholm:** None. **J. Guo:** None. **K.D. Hall:** None. **A. Martin:** None. **W.K. Simmons:** None.

Poster

757. Human Reward

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Topic: F.03. Motivation and Emotion

Support: Natural Science Foundation of China (31000502)

Title: Task relevance regulates the interaction between reward expectation and the processing of emotional word: Electrophysiological evidence

Authors: *P. WEI, D. WANG, L. JI;
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Abstract: In two electrophysiological experiments, we investigated the impact of reward expectation on the processing of emotional word using a cue-target paradigm while the event-related potentials (ERPs) were recorded. Both experiments employed a 2 (cue type: incentive vs. non-incentive) × 2 (emotional content of the target word: negative vs. neutral) within-participant design. A cue indicating the reward condition of each trial (incentive vs. non-incentive) was followed by the presentation of a negative or neutral word, the target. Participants were asked to discriminate the emotional content of the target word in Experiment 1 but to discriminate the color of the target word in Experiment 2, rendering the emotional content of the target word as task-relevant in the former but task-irrelevant in the latter. Behavioral results from Experiment 1 showed faster reaction times (RTs) in the incentive condition than in the non-incentive condition, and faster RTs to negative target word than to neutral word, demonstrating the effect of reward on facilitating task concentration and the effect of negative word capturing attention. Cue-

elicited ERPs were modulated by reward expectation, such that the incentive cue elicited larger N1, P2 and P3 amplitudes than the non-incentive cue. Target-elicited P2, P3, N400 and early posterior negativity (EPN) components all showed larger average amplitudes for the negative word than for the neutral word. Importantly, P3 and EPN components showed the interaction between reward condition and the emotional contents of the target word, such that the difference between average amplitudes for negative and for neutral words in the incentive condition was significantly larger than that in the non-incentive condition. Behavioral results from Experiment 2 showed faster RTs in the incentive condition than in the non-incentive condition. Cue-elicited ERPs showed the same pattern as the results in Experiment 1. Target-elicited EPN showed larger averaged amplitude for the negative word than for the neutral word. P3 component showed the interaction between reward condition and the emotional content of the target word, with differential responses to negative and neutral words in the non-incentive condition but no difference in the incentive condition. These results indicated that reward expectation improves top-down attentional concentration to task-relevant information, with enhanced sensitivity to the emotional content of the target words when the emotionality was task-relevant but with reduced differential brain responses for emotional words when it was task-irrelevant.

Disclosures: **P. Wei:** None. **D. Wang:** None. **L. Ji:** None.

Poster

757. Human Reward

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Topic: F.03. Motivation and Emotion

Support: VR grant

Title: Seeking pleasant touch: Neural correlates of behavioral preferences for skin stroking

Authors: ***I. PERINI**, H. OLAUSSON, I. MORRISON;
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Abstract: Affective touch is a dynamic process. In this fMRI study we investigated characteristics of touch pleasant touch by looking at their effects on behavior. We delivered soft brush strokes at different velocities on arm and palm skin, using a novel feedback-based paradigm in which participants could choose whether the caressing speed they would receive in a given trial would be the same as or different than the preceding trial. In every trial, tactile

stimulation was followed by a button-press in which the subject chose whether to repeat that stroking speed ("repeat") or change to another, randomly-selected speed ("change"). Since preferred stroking speeds should be sought with greater frequency than non-preferred speeds, this paradigm provided a measure of such preferences in the form of active choices. The stimulation velocities with respect to response properties of (CT) afferent nerves in the skin, the mean firing rate of which shows a linear correlation with pleasantness of stroking and stroking velocity (Loken et al., 2009). The behavioral findings demonstrated that gentle, dynamic stimulation optimal for activating CT-afferents not only affected behavior, but engaged brain regions involved in reward-related behavior and decision-making. This was the case for both hairy skin of the arm, where CTs are abundant, and glabrous skin of the palm, where CTs are absent. Participants preferred arm stroking at CT-optimal speeds of 1, 3, and 10 cm/s, and palm stroking at 3 cm/s, choosing to repeat rather than change away from these speeds significantly more often. The fMRI results revealed that after the sensory stimulation but before button-press indication of choice, anterior insula was strongly activated. A critical aspect of this interval was revealed by contrasting all "repeat" with all "change" trials. The areas showing selective activation for repeats versus changes were in the precentral gyrus, left dorsolateral prefrontal cortex (dlPFC) and the head of the caudate, areas associated with goal-directed behavior and reward expectancy. The percentage of repeat to change trials for each stroking speed formed a binomial distribution; speeds which fell above this distribution's chance-level likelihood of choosing "repeat" were considered preferred stimuli. The resulting contrast of all preferred vs non-preferred speeds_regardless of skin type_revealed activation in dlPFC alongside posterior insula, a main cortical target of the CT afferent pathway. The posterior insular and dlPFC activations therefore suggest that these areas reflect value-based preferences among tactile brush strokes at different stimulation speeds.

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Poster

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Topic: F.03. Motivation and Emotion

Support: NIH UL1TR000001

NIH S10 RR29577

Title: Acute exercise increases cognitive reappraisal in light to moderate smokers

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Abstract: Tobacco use is the top preventable cause of death in the United States. Acute exercise has been shown to alter brain responses to smoking cues and reduce cue-reactivity and craving in moderate to heavy smokers. However, little is known about the effects of acute exercise on reducing craving and cue-reactivity in light to moderate smokers. The current pilot study examined the impact of 30 minutes of acute exercise on craving and brain responses to smoking cues in light to moderate smokers (1-12 cigarettes/day). Thirteen smokers were recruited to participate in two functional magnetic resonance imaging (fMRI) appointments (counterbalanced). For each fMRI scanning session participants viewed images of smoking related cues (e.g. pack of cigarettes) and images of non-smoking related cues (e.g. pack of pencils). During one appointment smokers completed 30 minutes of exercise immediately before an fMRI scanning session and during the other appointment smokers completed 30 minutes of sedentary activity immediately before an fMRI scanning session. The results showed reduced activation immediately following exercise in the dorsolateral prefrontal cortex when smokers viewed images of smoking related cues compared to non-smoking related cues. Trends were found for reductions in self-reported craving immediately following exercise ($p = .10$) and in anterior cingulate (ACC) activations to smoking cues. Together these pilot data indicate that acute exercise is associated with increased activation in cognitive reappraisal brain regions during cue-reactivity and a pattern of reduced craving. Overall this project will inform future studies aimed at combining exercise with smoking cessation interventions by providing evidence about how exercise impacts brain responses to smoking cues in the environment.

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Poster

757. Human Reward

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Topic: F.03. Motivation and Emotion

Support: CAPES

CNPq

Title: Effect of emotions generated by events of life on food intake in women

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Abstract: Evidence from many studies suggest that emotions affect food consumption. In modern life, we are exposed to a variety of sweet foods that have hedonic impact, so it is important to understand how sweet food consumption is affected by specific emotions. The objective of this study was to investigate the influences of negative emotions triggered by life events on calories intake and healthy sweet food and unhealthy sweet food consumption by overweight and normal weight women. It was an experimental study with mixed methodology: we used analogic scales to measure emotion before and after videos interventions (VI) and focus group methods after the experiment. A sample of 43 women was divided into 2 groups, 23 with overweight and 20 with normal weight. Each group participated in two VI: one video that evoked emotions aroused by life dramas (intervention with emotions of real life - IERL) and another with common everyday scenes, considered as intervention with neutral emotions (INE). After the VI, the women were offered ad libitum mid-morning snack meals containing healthy sweet foods (HSF) and unhealthy sweet foods (USF). The amounts of these two food types ingested by the women and their respective calories were measured. After the INE, the calories and amount of USF intake by the overweight women were, respectively, 39% and 47% higher than the consumption by the normal weight group. Both groups increased significantly the calories intake and the USF consumption after the IERL in comparison with the INE. Normal weight women had increases of 82% in USF consumption and 51% in calories intake, while overweight woman had increases of 48% in USF consumption and 39% in calories intake. Contrary to the expectations, the increases in the normal weight group were higher than the increases in the overweight group. During the IERL, the amounts of calories intake and USF consumption of the women from the overweight group were equated by those of the women from the normal weight group. There was no statistical increase in the consumption of HSF between interventions, nor significant difference between groups. Our results suggest that negative emotions of real life can trigger increases in calories intake and also the consumption of USF.

Disclosures: R.W. Diez-Garcia: None. **A.C. Aguiar-Moreira:** None.

Poster

757. Human Reward

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Topic: F.03. Motivation and Emotion

Support: French Délégation Générale de l'Armement

European Research Council Starting Grant

Title: "Money is so crude": Dissociable roles of ventral striatum and ventromedial prefrontal cortex in the construction of subjective value

Authors: *A. LOPEZ[1,2,3], S. BOURGEOIS-GIRONDE[2,4], S. BOURET[1], M. PESSIGLIONE[1,3]; [1]Motivation, Brain and Behavior Team, Inst. Du Cerveau Et De La Moelle Épinière, Paris, France; [2]Inst. Jean-Nicod (ENS-EHESS), Ecole Normale Supérieure, Paris, France; [3]Univ. Pierre et Marie Curie, Paris, France; [4]Lab. d'Economie Moderne, Univ. Paris 2, Paris, France

Abstract: Basic choice models conceive decision-making as a two-step process, with first valuation of available options and then selection of the best option. Recent neuroimaging studies have identified the ventromedial prefrontal cortex (vmPFC) and the ventral striatum (vS) as the two main structures of the human brain valuation system, but their respective role is still a matter of debate. One dissociation present in the literature is that simple effort tasks, such as squeezing a handgrip to get a monetary reward involves the vS but not the vmPFC. In contrast, the vmPFC is consistently implicated in rating and choice tasks (with or without the ventral striatum). However, it remains unclear whether the dissociation relates to the task or to the stimulus. Indeed, choice and rating tasks necessitate items that are more complex than the monetary incentives used in effort tasks, otherwise they would be trivial. These tasks typically use multidimensional goods: food, faces, paintings, trinkets, lotteries etc. To clarify this issue, we investigated value encoding in a classical effort task using a complex reward. Our hypothesis was that the vmPFC participates in the valuation process whenever several dimensions need to be integrated, even if this valuation process serves the purpose of an effort task. We used functional magnetic resonance imaging (fMRI) to measure neural responses to compound incentives that participants could earn by performing an effort task. More precisely, the force exerted on a handgrip, relative to the subject's maximal force, determined the probability of winning the compound incentive, which was composed of two monetary amounts, one for the subject (personal gain) and one for a charity (altruistic donation). These amounts were varied across trials such that we could search for the neural correlates of their aggregated value, inferred from the pattern of force produced. We found in this situation, the vmPFC did encode the incentive value that was driving the effort, together with the vS. We therefore conclude that the previously observed dissociation was not due to the task (effort versus choice or rating), but to

the stimuli being too simple (monetary incentives). More generally, we suggest that the vmPFC is recruited when valuation needs integration of several dimensions, and that the vS might be sufficient in the other cases.

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Poster

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Topic: F.03. Motivation and Emotion

Title: Differential brain activation induced by cocaine vs. heroin-related emotional imagery as a function of context: an fMRI study in drug addicts

Authors: *S. DE PIRRO[1,2,3], G. GALATI[4,5], A. BADIANI[1,2,3];
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Abstract: Introduction: Epidemiological and clinical evidence indicates that environmental factors play a central role in modulating drug reward and drug abuse in humans and that this influence is substance-specific (Kendler et al. 2003; Badiani 2013). Heroin and cocaine co-abusers, for example, report distinct preferred settings for the two drugs. Heroin is used preferentially at home whereas cocaine is used preferentially outside the home (Caprioli et al. 2009; Badiani and Spagnolo 2013). The aim of the present study was to investigate the neurobiological basis of drug-setting interaction in human addicts by coupling drug-related emotional imagery with functional Magnetic Resonance Imaging (fMRI). Methods: Twenty addicts with a history of heroin and cocaine co-abuse were recruited at the addiction clinic Villa Maraini in Rome (Italy). Functional MRI scans were collected during an emotional script-based imagery procedure aimed at recreating different contexts of drug taking. The participants were asked to imagine taking heroin or cocaine either at home or in non-domestic setting, such as pubs or clubs. Craving, vividness and subjective pleasure were assessed using visual analogue scales. Results: Whole brain analyses using $p<0.05$ correction for multiple comparisons revealed positive BOLD signal changes in the caudate, right temporal gyrus (BA20-21), left inferior frontal gyrus (BA45), left middle frontal gyrus (BA46), posterior cingulum (BA23),

supplementary motor area (BA32), and cerebellum during drug-related imagery (taking heroin or cocaine either at home or outside the home) relative to the baseline (relaxing either at home or outside the home). Significant interactions between drug and environment were found in the right ($F(1,19) = 5.5$; $p=0.03$) and left ($F(1,19)=9.34$; $p=0.006$) cerebellum, as well as in the BA46 ($F(1,19)=8.9$; $p=0.008$). In these regions, there were greater increases in BOLD signal when the subjects imagined taking cocaine at home and heroin outside the home ('least preferred' conditions) than when they imagined taking cocaine outside the home and heroin at home ('most preferred' conditions). Conclusion: The present results confirm that the affective response to addictive drugs is modulated in a substance-specific manner by the surroundings of drug taking and suggest that the dorsolateral prefrontal cortex and cerebellum may be implicated in this interaction. This finding is particularly interesting in the light of a previous study by Sabatinelli and colleagues (2006) showing activation of the prefrontal cortex and cerebellum during emotional imagery.

Disclosures: **S. De Pirro:** None. **G. Galati:** None. **A. Badiani:** None.

Poster

757. Human Reward

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Program#/Poster#: 757.21/VV33

Topic: F.03. Motivation and Emotion

Title: A baby's smile increases cortical eeg correlations in both biological and adoptive mothers

Authors: *R. M. HIDALGO AGUIRRE[1], M. PÉREZ HERNÁNDEZ[2], C. AMEZCUA GUTIÉRREZ[2], M. GUEVARA PÉREZ[2], M. HERNÁNDEZ-GONZÁLEZ[2];
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Abstract: Maternal behavior involves those care-related behaviors that females display towards immature offspring which contribute to their development and increase their chances for survival. It has been reported that the sensory stimuli emitted by newborns can regulate and maintain maternal behavior, and that birth mothers show varying degrees of brain activation in relation to visual and auditory stimulation from infants that might reflect sensory processing and/or their own emotional states. Women who adopt babies show caring behaviors and responses to stimuli that are similar to those performed by biological mothers. The aim of this study, therefore, was to explore whether certain visual stimuli emitted by babies -smiling and crying- are related to variations in the correlation of cortical electroencephalographic activity

(EEG) in biological and adoptive mothers. The participants were 30 women aged 25-to-45; 10 were biological mothers (BM); 10 adoptive mothers (AM); and the other 10 nulliparous women (i.e., not mothers [NM]). EEGs were recorded from the frontal, parietal and temporal areas under two conditions: at rest with eyes open; and during observation of videos without sound of a baby, also under two conditions -crying and smiling- and a neutral video of colored, moving waves synchronized with a melody. The two groups of mothers, BM and AM, showed similar increased correlation patterns in relation to the visual stimuli, which were not present in the NM group. While watching the video of the smiling baby, BM and AM showed higher interhemispheric correlations (rTER) among all derivations and an increased frontoparietal intrahemispheric correlation (rTRA) at the fast frequencies. In addition, AM presented a higher rTER in the alpha and beta bands while watching the video of the baby crying, but only in temporal derivations. These EEG data show that smiling induces a higher degree of coupling than crying among the cortices of the biological and adoptive mothers, and allows us to suggest that the brain functionality of BM and AM is similar, but not completely equal when they are exposed to visual stimuli emitted by babies.

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Poster

757. Human Reward

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Topic: F.02. Animal Cognition and Behavior

Title: An attempt to dissect fundamental brain functions in decision-making task

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Abstract: Decision-making is a higher brain function that is organized by several fundamental brain functions (memory retrieving, evaluation of each possible event, selection of event, updating of memory, motor planning etc.) and many brain areas. One problem in traditional decision-making tasks is that the results of decision-makings were output as motor actions. Because of this problem, it is not possible to discriminate each fundamental brain function and

identify the functional roles of brain circuits for each function. To address this issue, we adopted two tasks to segregate a process of motor planning and process of selection of event from traditional decision-making task. Subjects were trained to gaze a target in the screen (task 1) or push a button that corresponds to the location of the target (task 2). One target was chose from two. Each target delivers rewards at different probability. This design enables us to segregate the process for selection of event in both task and the process for motor planning in task1. As the first stage of this study, we have showed a recording of skin conductance can be used to detect cognitive excitation. Rewarding probability dependent skin conductance responses were observed in some experimental conditions. These results imply that the recording of skin conductance can detect cognitive excitation and it can be used to dissect the fundamental brain functions in the future. But considerable day-to-day fluctuations of the responses are also observed. Therefore further modification of task, experimental condition and analyses might be required.

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Poster

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Topic: F.03. Motivation and Emotion

Support: Wellcome Trust 098282

Title: Neural basis for behavioral apathy in humans

Authors: V. BONNELLE[1], S. MANOHAR[1], T. BEHRENS[2], *M. HUSAIN[3];
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Abstract: Apathy is a common syndrome observed in many brain disorders, but also to some extent in the normal population, where it contributes to poor education outcomes and lack of success in gaining employment. The neural basis, whether structural or functional, for individual differences in apathy traits in the non-pathological population are however not well understood. Deciding whether an action is worth taking is likely to be a key element in the expression of apathy. Here, we investigated whether individual differences in apathy trait are associated in the recruitment of neural systems involved in effort- and reward- based decision-making. We used a

task where subjects (N=37) have to decide whether or not they want to engage in a physically effortful response (hand grip) given a particular reward and effort requirement. Apathy scores were measured using a modified version of the Lille Apathy Rating Scale, suitable for use in a non-clinical population. We focused on the Action Initiation subscale of the LARS-e, which indexes behavioral aspects of apathy such as initiative and every-day productivity. More apathetic individuals (i.e. those with lower Action Initiation scores) were more sensitive to physical effort, which was associated with stronger activation in brain regions involved in effort discounting such as the nucleus accumbens and the mid-cingulate cortex. Critically, apathy traits were strongly related to both structural and functional differences within the medial frontal wall: More apathetic individuals showed increase recruitment of brain regions involved in motor preparation such as the cingulate and supplementary motor area, and reduced structural integrity of the anterior cingulum bundle. Inefficient communication between the anterior cingulate and motor preparation systems may cause suboptimal response anticipation and preparation, associated with increased recruitment of the systems involved and difficulty initiating actions in apathetic individuals.

Disclosures: **V. Bonnelle:** None. **M. Husain:** None. **S. Manohar:** None. **T. Behrens:** None.

Poster

757. Human Reward

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 757.24/VV36

Topic: F.03. Motivation and Emotion

Support: Enhanced Nathan Kline Institute-Rockland

Title: Brain network functional connectivity is disrupted in childhood obesity

Authors: B. A. CHODKOWSKI[1,2], K. D. NISWENDER[3,4], *R. L. COWAN[1];
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Abstract: Background: Little is known about differences in brain network functional connectivity in childhood obesity. Ubiquitous media-driven exposure to visual images of food drives excess consumption of readily available high-calorie, highly palatable food. To maintain healthy weight, emotional drive (driving overconsumption) must be balanced by cognitive

control (controlling overconsumption). We hypothesize that obesity is due to a disruption in the functional connectivity within feeding-related brain networks mediating emotional drive and cognitive control. **Methods**: We acquired resting state fMRI data from 16 children (9 girls; mean age = 11.7 (1.5), range = [8.4, 13.9] yr; mean BMI% = 57.9 (29.1), range = [7, 96]%) from the Enhanced Nathan Kline Institute-Rockland Sample dataset. We used seed-based functional connectivity (FC) to analyze the functional organization of a feeding-related brain network. Our network includes the nucleus accumbens (NAc) as a hub that integrates inputs from the lateral orbitofrontal cortex (latOFC), associated with emotional drive, and the anterior cingulate cortex (ACC), associated with cognitive control. We quantified FC via partial correlation, and balance via an asymmetry measure computed from the partial correlation coefficients between latOFC:NAc (r_{drive}) and ACC:NAc ($r_{control}$): $balance = (r_{drive} - r_{control}) / ((r_{drive} + r_{control}) / 2)$. An increase in *balance* indicates an increase in emotional drive relative to cognitive control. We then determined the relationship between brain network FC and balance with waist-to-hip ratio. An increasing waist-to-hip ratio indicates increasing obesity. **Results**: We discovered that increasing imbalance in network FC is associated with increasing obesity. As waist-to-hip ratio increases, the FC between latOFC:NAc (emotional drive) increases relative to the FC between ACC:NAc (cognitive control) (p=0.04; R^2=0.26). This imbalance is driven by an increase in FC between latOFC:NAc (p=0.02; R^2=0.31) whereas the FC between ACC:NAc does not simultaneously keep pace (p=0.33; R^2=0.07). Thus, as obesity increases, so does imbalance. **Conclusions**: Our results reveal that greater functional connectivity between a network region associated with emotional drive and the NAc relative to functional connectivity between a network region associated with cognitive control and NAc is related to increasing obesity. Because intensive dieting in adults does not lead to long-lasting weight loss, understanding the neurobiological basis of childhood obesity is critical to treatment and prevention efforts.

Disclosures: B.A. Chodkowski: None. **K.D. Niswender:** 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.; Novo Nordisk. **R.L. Cowan:** 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.; Novo Nordisk.

Poster

757. Human Reward

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 757.25/VV37

Topic: F.03. Motivation and Emotion

Title: Neural dissection of incentive and hedonic processes depicts a regulation mechanism of reinforcement driven behavior in humans

Authors: *T. GONEN[1], E. ELDAR[2], G. RAZ[3], T. HENDLER[3];
[1]Functional Brain Center, Tel Aviv Med. Ctr., Tel Aviv, Israel; [2]Princeton Neurosci. Institute,Princeton Univ., Princeton, NJ; [3]Functional Brain Center, Tel Aviv Sourasky Med. Ctr., Tel Aviv, Israel

Abstract: Reinforcements signal both incentive and hedonic values (rewarding/punishing and appetitive/aversive, respectively). The integration of these two values often raises a conflict considering the preferable behavioral choice (approach/avoidance). However, the way they interact in order to guide goal-directed behavior is yet to be revealed. The Reinforcement Sensitivity Theory assumes that a Behavioral Inhibition System (BIS), and specifically its node in the hippocampus, has a key role in regulating reinforcement based choices according to their values. Here we aimed to dissect the neural processes of motivational values and to portray their dynamic interaction on-line during choice behavior. 46 healthy subjects were fMRI scanned while playing a novel computer game that induces motivational behavior. Controlled and uncontrolled rewards and punishments allowed for dissociation of incentive and hedonic elements of reinforcement. Whole brain functional connectivity (FC) driven by the hippocampus was conducted to investigate the BIS's role in regulating motivational choices. The contrast of controlled vs. uncontrolled events revealed an incentive element, including areas of action; M1, SMA, and arousal; hypothalamus, PAG, vs. an evaluative element which resembled mostly the Default Mode Network including the hippocampus. The contrast of reward vs. punishment revealed expected hedonic activation in NAC and PAG & insula, respectively. Conjunction was found between the incentive and punishment network in hypothalamus, PAG, and motor cortices; as well as between the evaluative and reward network in NAC, hippocampus and vmPFC. Hippocampus was co-activated with another major node of the BIS, the vmPFC; as well as with hedonic related areas: PAG and VTA; however it was also co-activated with incentive related areas: amygdala and hypothalamus. Important, the hippocampus-vmPFC connection was also related to rate of approach and avoidance moves in the game. Using an ecological dynamic paradigm we delineated the interacting neural processes that underlie motivational values; incentive and hedonic. We further portrayed a regulation system which resembled the BIS, which co-activated with regions involved in both incentive and hedonic value processing, suggesting the importance of integration. This demonstrates that the regulation of motivational behavior is accomplished by coordinated linking of the incentive and hedonic components of motivation.

Disclosures: **T. Gonen:** None. **E. Eldar:** None. **G. Raz:** None. **T. Hendler:** None.

Poster

757. Human Reward

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 757.26/VV38

Topic: F.03. Motivation and Emotion

Support: The Wellcome trust (098282/Z/12/Z)

Title: Subjective valuation of rewards discounted by cognitive and physical effort

Authors: *M. A. APPS[1], T. T.-J. CHONG[2], L. GRIMA[1], K. GIEHL[1], M. HUSAIN[2]; [1]Exptl. Psychology, [2]Nuffield Dept. of Clin. Neurosci., Univ. of Oxford, Oxford, United Kingdom

Abstract: Motivation is underpinned by cost-benefit based decisions. Previous studies have shown that rewards (benefit) are devalued by the *physical* effort (cost) of actions. Yet, we often make choices between options that differ in *cognitive* demands but have equitable *physical* costs. Here, we examined how healthy human adults subjectively value rewards when faced with varying cognitive and physical demands. We used two tasks which aimed to keep physical and cognitive demands equivalent overall. (1) *Cognitive*. Subjects (*N*=30) made switches of attention between peripheral locations whilst maintaining central fixation (see Yantis et al., 2002, *Nature Neuroscience)*. They were required to make a button press when detecting a target cue in the peripheral location. (2) *Physical*. Subjects had to maintain a sustained force by squeezing a handheld dynamometer. We parametrically varied the demands of each task, by varying the number of attentional switches (1, 2, 3, 4, 5,or 6 switches) in the *cognitive* task, and the force level in the *physical* task (8, 13, 18, 23, 28, 33% of the maximum force they could achieve). After training, subjects made choices between a *fixed* low-reward (1 credit) , low-effort option and a *variable* higher effort, higher reward (2, 4, 6, 8, 10 credits) option. Choices were made on separate trials for the *cognitive* and *physical* tasks. Both *physical* and *cognitive* effort devalued rewards, with choices of the fixed option increasing as the effort level of the variable option increased. Computational modelling of choices revealed that cognitive and physical effort exponentially discounted rewards. There was no overall difference in discounting rates between cognitive and physical effort, suggesting the two tasks were matched in terms of demand. However, there were significant individuals differences, with subjects discounting more on one task than the other. Our results characterize the mechanisms by which cognitive and physical effort discount rewards and highlight the subjectivity of each effort domain's influence on motivation. These findings pave the way for future studies examining the neural basis of

subjective effort discounting – for physical and cognitive demands – and the mechanisms that underpin disorders of motivation, such as apathy.

Disclosures: **M.A. Apps:** None. **T.T. Chong:** None. **L. Grima:** None. **K. Giehl:** None. **M. Husain:** None.

Poster

757. Human Reward

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 757.27/VV39

Topic: F.03. Motivation and Emotion

Support: NRF of Korea, 2012R1A2A2A01012159

Title: Neural responses during reward and avoidance learning in people with excessive internet use

Authors: H. YOON, H. M. AHN, *S. KIM;
Brain and Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: As excessive internet use (EIU) has been associated with various physical and mental health problems, the need to understand its underlying cognitive and neural basis has rapidly increased. One of the characteristic features identified in people with EIU is increased sensitivity to reward and decreased sensitivity to punishment. To investigate neural basis of differences in sensitivity to reward and punishment in people with EIU, we conducted an fMRI study where adults with EIU (n=11, 25.45 yrs ± 3.32) and healthy controls (n=22, 23.91 yrs ± 2.52) performed a reinforcement task inside the brain scanner. In the task, each trial started with presentation of a pair of fractal stimuli and one of them was associated with a greater probability of winning monetary reward (reward trials) or with a greater probability of avoiding monetary loss (avoidance trials). Participants chose one of them and monetary feedback was provided after 4 seconds of anticipation period. Brain images were obtained using a 3T Siemens scanner located at the Korea University Brain Imaging Center. Images were analyzed using SPM 8. BOLD hemodynamic responses were modeled for the anticipation period (0 - 4 sec after selection). Overall participants showed increased activation in the striatum, insula and OFC during reward anticipation. During anticipation of loss avoidance, activation in the dorsal striatum, insula, OFC, and dorsomedial PFC was found. The EIU group relative to the control group showed increased activation in the right anterior insula and right ventrolateral PFC during

reward anticipation. On the other hand, during avoidance anticipation, the EIU group relative to control group showed decreased activation in the posterior insula, and somatosensory regions. Provided that the anterior insula is associated with emotional awareness and saliency detection, and the posterior insula with sensory perception, our imaging results support that EIU may experience greater emotional feeling or increased saliency for rewards yet may have reduced somatosensory representation of punishment. These results contribute to our understanding of neural mechanisms underlying EIU.

Disclosures: H. Yoon: None. **H.M. Ahn:** None. **S. Kim:** None.

Poster

757. Human Reward

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 757.28/VV40

Topic: F.03. Motivation and Emotion

Support: Utah State University

Title: Cooperation and strategy during social dilemmas: An EEG hyperscan analysis

Authors: *N. J. A. WAN[1], A. J. WILSON[2], B. S. ROBINSON[1], K. E. JORDAN[1];
[1]Psychology, Utah State Univ., Logan, UT; [2]Psychology, Western Illinois Univ., Macomb, IL

Abstract: Knowledge about the intricacies of cooperation between humans on a neural level is limited. Previous research concerning cooperation and defection decision-making processes has shown it is possible to predict acts of defection (but not always cooperation or strategy-based decisions) during the Prisoner's Dilemma (PD). Specifically, greater EEG beta (12 - 24Hz) and gamma (>25 Hz) activation about the dorsolateral prefrontal cortex (DLPFC), a region implicated in decision-making, indicates a defection choice. Using methodology from Kummerli et al. (2007) decision-making study on PD and a variant social dilemma game, Snowdrift, we investigated how predictive a cooperate-or-defect decision is on the neural level of hyperscanned EEG participants. We divide decisions into cooperation and defection epochs and also reaction based on outcomes. We then analyze DLPFC for recorded alpha (8 - 12Hz) and beta activities. Acts of cooperation are shown to be characterized by greater alpha activation than acts of defection about the DLPFC across all participants. Using a simple hyperscanning analysis, pairs who cooperate the most frequently had the most alpha activation about the DLPFC. Neural activation for the tit-for-tat strategy shows greater alpha activation than for other strategies,

irrespective of choice. Applying the neural efficiency hypothesis, those who cooperate more do so by using fewer neural resources (shown by greater alpha activation). In the future, such findings should be further investigated using populations who may show atypical development in cooperation and social interaction (e.g., ASD). References: Kümmerli, R. et al. (2007). Human cooperation in social dilemmas: comparing the Snowdrift game with the Prisoner's Dilemma. Proc. R. Soc. B, 274(1628), 2965-70.

Disclosures: **N.J.A. Wan:** None. **A.J. Wilson:** None. **B.S. Robinson:** None. **K.E. Jordan:** None.

Poster

757. Human Reward

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 757.29/VV41

Topic: F.03. Motivation and Emotion

Support: Lieber Institute for Brain Development

Intramural Research Program of National Institute of Mental Health, NIH

Title: Human striatal responses to reward void of motivation, and motivation void of reward

Authors: ***S. TILLEM**[1], L. M. PEPE[2], D. R. WEINBERGER[1], C. F. ZINK[1];
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Abstract: Cues that predict the availability of future reward elicit a cognitive appraisal of reward and hedonic response, as well as increase motivation to achieve reward. Neurally, reward-predicting cues evoke activation in the human ventral striatum (VS). In an attempt to accurately interpret the VS response to reward-predicting cues, recent studies have modulated the motivational and reward components of reward-predicting cues in opposite directions to demonstrate that the VS activity is more associated with the motivational salience surrounding these cues. These studies do not, however, examined the VS response to reward cues completely void of motivational salience, or motivational cues completely void of predictive reward value. Thus, despite evidence that the VS preferentially responds to motivational salience of the cues, the VS may still be involved in processing both reward value and motivational salience. In the current study, 48 healthy volunteers underwent 3T fMRI while performing two novel variants of

the Monetary Incentive Delay Task (MID) to examine VS responses to reward cues void of motivational salience and motivational cues void of predictive reward value. In Task 1, reward-predicting cues were made pure reward-related stimuli (void of motivational properties) by modifying the MID event order so reward-predicting cues were presented after target detection and before outcome (target-cue-outcome). Thus, reward outcomes were contingent on the subjects' actions, but the cue no longer motivated the upcoming behavior. In Task 2, the event sequence was similar to the traditional MID (cue-target-outcome), but cues did not predict reward availability above chance (cues predicted 50% reward/50% no reward, thus while reward is possible on each trial, whether the trial is rewarded or not cannot be predicted by the cues). Rather, the cues served as purely motivating stimuli by signaled that an upcoming target was imminent; both cues increased motivation to a similar extent, while being void of reward properties. The imaging data from each task were subjected to an event-related, random-effects, striatal ROI analysis ($p < 0.05$, FWE-corrected for multiple comparisons). In Task 1, neither reward cue nor nonreward cue activated the VS. In fact, the VS significantly deactivated relative to baseline following both cue types. In contrast, in Task 2, both motivation-related cues significantly activated the VS relative to the baseline to similar extents. These results are important to clarify the functionality of the VS in reward and motivation processes, and specifically point to a role of the VS in motivational processes and not reward properties.

Disclosures: **S. Tillem:** None. **L.M. Pepe:** None. **D.R. Weinberger:** None. **C.F. Zink:** None.

Poster

757. Human Reward

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 757.30/VV42

Topic: F.03. Motivation and Emotion

Support: NIH Grant R21 DA024401

Title: Frontal and limbic emotional reactivity are associated with different monetary incentive delay profiles

Authors: *J. E. JOSEPH, T. KELLERMANN, X. ZHU;
Dept. of Neurosciences, MUSC, Charleston, SC

Abstract: Motivation for receiving rewards or avoiding losses may be an important factor in vulnerability to substance abuse. In addition, altered emotional reactivity and regulation are key

components in substance dependence. This study examined whether these two aspects of behavior are associated at the neural level in non-dependent subjects. fMRI response to emotional pictures and response on a monetary incentive delay (MID) task were measured in adults (n=50) and adolescents (n=26). Regression analyses examined whether fMRI response to valence or arousal of the emotional stimuli could predict MID profiles in the right nucleus acumbens (NA), a region strongly implicated in reward processing. A general pattern that emerged across these regressions was that (a) higher response to emotional stimuli in the dorsal and lateral frontal cortex predicted reduced gain sensitivity and higher response to small values in the right NA, (b) higher response to emotional stimuli in limbic structures (amygdala and ventromedial prefrontal cortex) predicted enhanced gain sensitivity in the right NA, and (c) left NA response to negative emotional was associated with greater right NA response to small values, but (d) higher left NA response to positive pictures were associated with greater right NA response to large incentive values. In summary, these findings suggest that greater emotional reactivity in frontal regions is associated with attenuated reward processing but greater emotional reactivity in limbic regions is associated with enhanced reward processing. Hence, the relative recruitment of frontal versus limbic circuitry during emotional induction may be a critical factor in vulnerability to substance abuse.

Disclosures: J.E. Joseph: None. **X. Zhu:** None. **T. Kellermann:** None.

Poster

758. Sex and Steroids

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 758.01/VV43

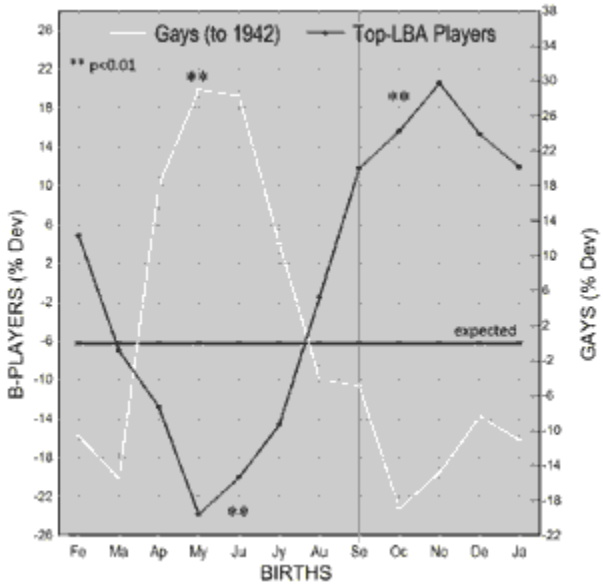
Topic: F.04. Neuroethology

Title: A shared rhythm of seasonal births among baseball players and lesbians with an opposite rhythm among gay men

Authors: *G. MARZULLO;
Per Aspera Res. Fndn., New York, NY

Abstract: Mid-20 century studies of schizophrenia (SCZ) found a disease incidence excess among people born around late-February and a deficit among those born six months later around late-August. Given that SCZ is associated with and may result from deficits of cerebral asymmetries, we previously investigated hand preferences and month of birth among professional baseball players. The results led us to a “solstitial” hypothesis implicating maternal

melatonin-mediated and other sunlight actions affecting left-right brain differentiation in the neurulating four-week-old embryo (Marzullo & Fraser, 2005, 2009; Marzullo & Boklage, 2011). Further studies of the same baseball players have now suggested that the same melatonin-mediated or other sunlight actions could also affect testosterone dependent male-female differentiation in the four-month-old fetus. Independently of hand-preferences, the baseball players (n=6,829), and particularly the stronger hitters among them, showed a unique birth seasonality with an excess around early-November and an equally significant deficit around early-May. In two smaller studies, American and other northern-hemisphere born lesbians showed the same strong-hitter birth seasonality while gay men showed the opposite seasonality. The sexual dimorphism-critical fourth-fetal-month testosterone surge coincides with the summer-solstice in early-November births and with the winter-solstice in early-May births. A "melatonin mechanism" is proposed based on these coincidences coupled with evidence that in seasonal breeders maternal melatonin imparts "photoperiodic history" to the newborn by direct inhibition of fetal testicular testosterone synthesis. The present effects were thus suggested to represent a vestige of this same phenomenon in man (Marzullo, 2014).



Disclosures: G. Marzullo: None.

Poster

758. Sex and Steroids

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 758.02/VV44

Topic: F.04. Neuroethology

Support: Department of Biological Sciences; MNSU Mankato

Undergraduate Research Center; MNSU Mankato

Title: The effect of steroid hormones on brain morphology in green anole lizards

Authors: S. A. GILBERT[1], P. S. CONNOLLY[2], B. A. GILBERT[3], *R. E. COHEN[4]; [1]Biol. Sci., [2]Chem., [3]Minnesota State Univ. Mankato, Mankato, MN; [4]Biol. Sci., Minnesota State University, Mankato, Mankato, MN

Abstract: Gonadal steroid hormones are important in maintaining reproductive behaviors, as well as brain morphology. Seasonally breeding animals, such as the green anole lizard (Anolis carolinensis) display dramatic seasonal changes in both brain morphology and reproductive behaviors. Anole lizards in the breeding season have an increased volume in brain regions that control reproductive behavior. Hormone levels also change seasonally such that testosterone (T) is produced in larger amounts during the breeding season. We tested the hypothesis that neural structures are maintained in the breeding season by steroid hormones. Breeding adult male anole lizards were gonadectomized and a capsule filled with T or left empty was inserted subcutaneously. One month later, the brain was collected, stained with thionin, and the volumes of three brain regions controlling reproductive behaviors were measured. T-treated (n = 5) green anole lizards had a larger preoptic area (POA) than control lizards (n = 3; t = 5.35, p = 0.002). Thus, T appears to be important in maintaining a larger POA volume during the breeding season. There was no effect of treatment on the medial amydgala (AMY; n = 3; t = 1.26, p = 0.275). This result was not surprising, as the AMY volume does not differ between unmanipulated breeding and non-breeding lizards. We also found no effect of treatment on the ventromedial hypothalamus (VMH; n = 2; t = 0.08, p = 0.9) volume, suggesting that T may not be a key factor in maintaining a breeding-like volume in this region. Next, we will determine whether T also affects the number of neurons in these regions, as well as whether T metabolites (dihydrotesoterone and estradiol) have similar effects on the AMY, VMH, and POA. Thus, our preliminary results suggest that steroid hormones are important in maintaining some of the breeding-like structures in the brain.

Disclosures: S.A. Gilbert: None. **P.S. Connolly:** None. **B.A. Gilbert:** None. **R.E. Cohen:** None.

Poster

758. Sex and Steroids

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 758.03/VV45

Topic: F.04. Neuroethology

Support: NIH Grant R01MH099085

Title: Sex differences in ketamine's antidepressant effects following social-isolation stress in C57BL/6 mice

Authors: *A. M. DOSSAT, K. N. WRIGHT, C. E. STRONG, M. KABBAJ;
Biomed. Sci., Florida State Univ., Tallahassee, FL

Abstract: In recent clinical trials, Ketamine, an NMDA receptor antagonist, showed great promise as a rapid-acting antidepressant. Despite this breakthrough, it is still not known whether or not there are sex differences in ketamine's antidepressant effects. Our group recently showed that female rats are more sensitive to the antidepressant effects of ketamine as compared to males, an effect that was mainly mediated by female gonadal hormones. In order to take advantage of genetic manipulations available in mice in future projects, the purpose of this study was to examine sex differences in ketamine's antidepressant effects following chronic social isolation (SI) stress in mice. In our protocol, adult C57BL/6 mice were either pair-housed (PH) or underwent 8 weeks of SI. To examine SI-induced anhedonia, anxiety- and depressive-like symptoms we used the sucrose preference test, novelty-suppressed feeding test (NSFT), and forced swim test (FST), respectively. Mice in SI did not display anhedonia at any time point examined. In the NSFT, acute ketamine treatment (3 mg/kg) significantly reduced latency to first bite in males in SI and in females in both SI and PH conditions, with females overall displaying longer latency to first bite compared to males. In the FST, ketamine reduced time immobile in both sexes. Interestingly, there was an interaction between housing conditions and sex. Indeed, PH males, but not males in SI, responded to ketamine treatment. PH females displayed a trend towards responding to ketamine while females in SI showed a significant response to ketamine. Taken together, our results provide evidence of a sex difference in ketamine's antidepressant effect in mice. We are currently examining neural activation following the stress of the FST, within brain areas associated with anxiety and depression to determine how this activation may be modulated by ketamine.

Disclosures: **A.M. Dossat:** None. **K.N. Wright:** None. **C.E. Strong:** None. **M. Kabbaj:** None.

Poster

758. Sex and Steroids

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 758.04/VV46

Topic: F.04. Neuroethology

Support: NSF HRD-1137725 (CREST)

Title: Contaminants found in Puerto Rican urban streams cause changes on general activity and agonistic behaviors of a native freshwater prawn species, Macrobrachium carcinus

Authors: *J. L. ORTIZ[1], N. RIVERA[2], M. SOSA[2];
[1]Anat. and Neurobio. Dept, Univ. of Puerto Rico, San Juan, Puerto Rico; [2]Anat. and Neurobio. Dept, Univ. of Puerto Rico Med. Sci. Campus, San Juan, Puerto Rico

Abstract: As natural landscapes are altered by human disturbances, the health of streams and their fauna are increasingly at risk. Urbanism in Puerto Rico is driving a continual conversion of land to anthropogenic uses, increasing the accumulation of contaminants in water resources. An increasing number of chemicals liberated into the environment through human activities have demonstrated potential for disruption of biological processes critical to normal growth and development of wildlife species. Crustaceans are major constituents of aquatic ecosystems, yet little is known about how anthropogenic chemicals affect important aspects of their behavior, such as locomotion and aggression, and the underlying neural circuitry. We are studying how organic and metal contaminants in urban rivers affect aggression and general activity of a local freshwater macro-invertebrate, Macrobrachium carcinus, the most prevalent prawn species found in the rivers of Puerto Rico. Phthalates, esters commonly used as solvents in personal care products and as additives to make plastic tubing and packaging materials more flexible, transparent and durable, as well as metal pollutants found in local urban streams were injected in the circulating hemolymph of adult male prawns at concentrations equal to the limits established by the EPA for drinking water. Fighting between prawn pairs were recorded and quantified by measuring specific parameters such as movement of claws, body posture, and position of legs to establish the level of aggression/dominance. Locomotion within a fixed-size enclosure was also tracked and quantified. Injection of low concentrations of dibutyl phthalate (DBP; 0.006 mg/L), chromium (Cr+3; 0.100 mg/L) and cadmium (Cd+2; 0.005 mg/L) significantly increased aggressive behavior (dominance index) and locomotion of adult male prawns. Besides increasing total distance travelled and time spent moving at a fast speed, injected prawns also ventured more often towards the center of the recording tank than they did under control conditions or when Ringer solution was injected. Comparison of pair encounters shows that DBP and Cd+2, but not Cr+3, injections in submissive prawns significantly decreased the number and duration of interactions and the number of interactions initiated by the dominant prawns. These results suggest that water contaminants associated with urbanism have an impact on prawn behavior and

locomotion, and it now remains to be determined how the corresponding neural function is affected at the circuit, cellular, molecular, and genetic levels.

Disclosures: **J.L. Ortiz:** None. **N. Rivera:** None. **M. Sosa:** None.

Poster

758. Sex and Steroids

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 758.05/VV47

Topic: F.02. Animal Cognition and Behavior

Support: Institute for Mental Health Research

NIH F32 MH093145

Title: Neurobehavioral effects of chronic antidepressant treatment in middle-aged rats: Sex-specific alterations in cognition and depressive-like behavior

Authors: ***R. HIROI**[1,2], A. M. QUIHUIS[1,2], C. N. LAVERY[1,2], S. J. GRANGER[1,2], G. WEYRICH[1,2], H. A. BIMONTE-NELSON[1,2];
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Abstract: Aging and the menopausal transition are each associated with affective disorders such as depression and anxiety, which is often co-morbid with cognitive impairment amongst elderly patients. Of note, women are more vulnerable to both cognitive and affective disorders than men. Antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs), are commonly prescribed to alleviate symptoms of depression and anxiety. Although the antidepressant effects of SSRIs in adults have been characterized, antidepressant and cognitive effects of SSRIs in the aged population are not well understood, especially regarding potential sex differences in efficacy. The current study investigated the effects of chronic SSRI citalopram administration on cognitive, anxiety-like, and depressive-like behaviors in male and female middle-aged rats, using the water radial arm maze (WRAM), open field test (OFT), and forced swim test (FST), respectively. We found that females outperformed males in working memory as tested on the WRAM, corresponding with and extending our previous sex difference findings on this task in young rats. The effects of chronic citalopram administration on cognition depended on memory domain; citalopram improved memory retention, despite the slower rate of learning during acquisition of the task for WRAM working memory. We also found that chronic citalopram had

sex-specific effects on depressive-like behaviors in the FST. These results suggest that factors such as age and sex play important roles in predicting the outcome of SSRIs on cognitive and affective behaviors. We are currently investigating potential underlying neurobiological mechanisms involving the midbrain serotonergic system. This study raises important considerations of sex when studying SSRI neurobehavioral effects, and warrants further investigations to decipher the distinct parameters in which mood- and cognitive- enhancing effects of antidepressants can be realized.

Disclosures: R. Hiroi: None. **A.M. Quihuis:** None. **C.N. Lavery:** None. **S.J. Granger:** None. **G. Weyrich:** None. **H.A. Bimonte-Nelson:** None.

Poster

758. Sex and Steroids

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State of Arizona

ADHS

Arizona Alzheimer's Disease Core Center

NIH Initiative for Maximizing Student Development program R25GM099650

NSF More Graduate Education at Mountain States Alliance

NSF Western Alliance to Expand Student Opportunities Louis Stokes Alliance for Minority Participation Bridge to the Doctorate cooperative agreement HRD-1025879

Title: The effect of varying 17β-estradiol treatment frequency on cognitive performance in middle-aged ovariectomized rats

Authors: *A. M. QUIHUIS[1,4], A. V. PRAKAPENKA[1,2,4,5], S. E. MENNENGA[1,4], R. HIROI[1,4], S. V. KOEBELE[1,4], R. W. SIRIANNI[3,5], H. A. BIMONTE-NELSON[1,4,5];
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AZ; [4]Arizona Alzheimer's Consortium, Phoenix, AZ; [5]Barrow Neurolog. Inst., Barrow Brain Tumor Res. Ctr., Phoenix, AZ

Abstract: There is accumulating evidence that, in both humans and rats, ovarian hormone loss contributes to cognitive decline, and that estrogen treatment can impact cognitive function. The factors impacting the realization and direction of estrogen's effects on the brain and its function are numerous. Temporal parameters of administration are likely critical to efficacy. The current study evaluated how the frequency of estrogen administration impacts cognitive performance in surgically menopausal rats. Ovariectomized middle-aged rats were subcutaneously injected with a vehicle treatment, or a daily, weekly, or bi-weekly (every other week) 17β-estradiol (E2) treatment. Rats were then tested on the water radial arm maze to test spatial working and reference memory, and the Morris water maze to assess spatial reference memory. Results indicated that any E2 treatment regimen was beneficial for spatial memory performance at some level, but with varied effectiveness. Notably, the daily E2 treatment group exhibited the best performance overall, especially during initial learning. E2 levels in blood serum, brain, and organs are currently being assayed to determine how varying the treatment regimen affects E2 levels present in the body, and how they relate to cognitive change. Results from this analysis will be employed to further study the effects of estrogen on cognitive performance utilizing brain-targeted nanoparticles as the delivery platform.

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Poster

758. Sex and Steroids

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State of Arizona

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Title: Cognitive changes across the menopause transition in a rat model: A longitudinal evaluation of the impact of age and ovarian status on spatial memory

Authors: *S. V. KOEBELE[1,2], S. E. MENNENGA[1,2], R. HIROI[1,2], L. T. HEWITT[1,2], A. M. QUIHUIS[1,2], M. L. POISSON[1,2], L. P. MAYER[3], C. A. DYER[3], H. A. BIMONTE-NELSON[1,2]; [1]Psychology, Arizona State Univ., Tempe, AZ; [2]Arizona Alzheimer's Consortium, Phoenix, AZ; [3]SenesTech Inc., Flagstaff, AZ

Abstract: Aging and the menopause transition are both intimately linked to cognitive changes during mid-life and beyond. Clinical literature suggests that the age at which women experience the onset of menopause impacts cognitive abilities later in life. The undoubtedly complex relationship among physiological, behavioral, and brain changes that occur during the natural transition to menopause, which is typically comorbid with aging, has only begun to be explored. To better understand these relationships, and to elucidate the trajectory of change with follicular depletion and aging, we employed a rodent model of transitional menopause utilizing the industrial chemical 4-vinylcyclohexene diepoxide (VCD), which selectively depletes ovarian follicles, allowing for retention of post-menopausal ovarian tissue. Here, ovary-intact Young and Middle-Aged Fischer-344 rats received either VCD or Vehicle treatment, and were repeatedly tested on the water radial arm maze spatial working and reference memory task over a six month span to assess the cognitive effects of transitional menopause via VCD-induced follicular depletion over time, as well as potential interactions with age. Both age and menopause status interacted with performance on spatial memory. Ongoing analyses of serum hormone levels, brain, and ovarian tissue will provide insight into behavioral correlates. The observed interaction of age and treatment with performance over time merits further research in order to determine the impact of each of these factors on spatial memory learning and maintenance during aging.

Disclosures: S.V. Koebele: None. **S.E. Mennenga:** None. **R. Hiroi:** None. **L.T. Hewitt:** None. **A.M. Quihuis:** None. **M.L. Poisson:** None. **L.P. Mayer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Senestech Inc. **C.A. Dyer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Senestech Inc.. **H.A. Bimonte-Nelson:** None.

Poster

758. Sex and Steroids

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More Graduate Education at Mountain States Alliance (NSF)

Western Alliance to Expand Student Opportunities Louis Stokes Alliance for Minority Participation Bridge to the Doctorate (NSF) Cooperative Agreement HRD-1025879

Title: A sensitive window for hormone therapy initiation during transitional menopause: Conjugated equine estrogens impair memory when given after, but not before, ovarian follicular depletion

Authors: *S. E. MENNENGA[1,2], J. I. ACOSTA[1,2], A. N. GARCIA[1,2], L. T. HEWITT[1,2], M. L. KINGSTON[1,2], C. W. S. TSANG[1,2], B. W. CAMP[1,2], L. MAYER[3], C. DYER[3], H. A. BIMONTE-NELSON[1,2];
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Abstract: Menopause is characterized by loss of circulating ovarian-derived estrogen and progesterone. Many women take hormone therapy (HT) to alleviate some symptoms of menopause; conjugated equine estrogens (CEE, tradename Premarin) are the most commonly prescribed estrogen component of HT in the US. In the rat, our and other laboratories have shown that CEE benefits spatial working and reference memory performance following surgical ovarian hormone loss via ovariectomy (Ovx), while we have shown that CEE impairs performance when administered post ovarian follicular depletion and after concomitant transitional hormone loss induced by 4-vinylcyclohexene diepoxide (VCD) administration. Several human studies have given rise to the critical window hypothesis, suggesting that there exists a window of time around menopause during which HT can benefit memory. Research in the rat model is in accordance with these findings, and has allowed methodical evaluation of critical window effects relative to abrupt hormone loss via surgical menopause; for example (Daniel et al., 2006), in middle-aged rats, 17B-estradiol given immediately, but not 5 months after, Ovx enhanced working memory, and place recognition. The goals of the present study

were to determine whether the cognitive impact of CEE HT is influenced by the timing of treatment initiation relative to follicular depletion; indeed, the prior study testing CEE effects in the transitional VCD model tested efficacy after follicular depletion. The current study replicated our prior findings showing that CEE treatment initiated after follicular depletion impairs performance on spatial working memory and does not impact performance on spatial reference memory. Additionally, we found that initiation of CEE HT earlier during follicular depletion reversed impairments induced by post-depletion administration when given for a short-term, but not a long-term, duration. CEE administration at any timepoint relative to follicular depletion, and at both durations, resulted in a transient improvement in learning a short-term memory task, not evident by the end of testing. The current results indicate that timing of CEE, the most commonly used estrogen component of hormone therapy, impacts cognitive efficacy, with administration after follicular depletion showing a more detrimental profile than if given earlier in follicular depletion. Hormone assays, ovary profiles, and brain processing are underway to further assess mechanisms of these effects.

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Poster

758. Sex and Steroids

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Topic: F.02. Animal Cognition and Behavior

Support: NIH P50 AT006268

Title: The effects of the botanical estrogen isoliquiritigenin (ISL) on cognition

Authors: *P. KUNDU[1], S. NEESE[2], S. BANDARA[1], S. MONAIKUL[1], W. G. HELFERICH[1], S. SCHANTZ[1];
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Abstract: Age-related declines in cognitive function can reduce speed of processing, working memory, and alter attentional resources. In particular, menopausal women may show an

increased vulnerability to brain diseases as well as an acceleration in the rate of cognitive decline, suggesting that estrogens may play a neuroprotective and neurotrophic role in the brain. To treat menopausal symptoms, many women turn to botanical estrogens that are promoted as a safe and natural alternative to traditional hormone replacement therapies. However, the majority of these compounds have not been systematically evaluated for efficacy and safety. This study investigated the efficacy of the commercially available botanical estrogenic compound isoliquiritigenin (ISL) to alter performance on an operant working memory task, delayed spatial alternation (DSA). ISL is a compound found in licorice root that has been shown to have a wide range of effects on different biological systems, including estrogenic properties. It is currently being used in over the counter dietary supplements. Middle-aged (12-month old) Long-Evans female rats were ovariectomized and orally dosed with either a 0 mg, 6 mg, 12 mg or 24 mg treatment of ISL 60 minutes before testing on the DSA task. The DSA task required the rat to alternate its responses between two retractable levers in order to earn food rewards. Random delays of 0,3,6,9 or 18 seconds were imposed between opportunities to press. ISL failed to alter DSA performance. Previous research has found that estrogenic compounds, including the botanical estrogen genistein impair performance on the task. The goal of our botanical estrogens research is to find compounds that offer some of the beneficial effects of estrogen supplementation, without the harmful effects. Given that no detrimental effects of ISL were observed on the DSA task, we are currently testing ISL to see if it has the ability to enhance performance on a hippocampally mediated task. Supported by NIH P50 AT006268.

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Poster

758. Sex and Steroids

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant RO1AG037984

NIH Grant RO1AG036800

Evelyn F. McKnight Brain Research Foundation

Title: Epigenetic regulation of estrogen receptor α contributes to age-related differences in transcription across the hippocampal regions

Authors: *L. IANOV, A. KUMAR, T. C. FOSTER;
McKnight Brain Institute, Univ. of Florida, Gainesville, FL

Abstract: An extended period of estradiol (E2) deprivation results in a decline in E2 responsiveness in the hippocampus, including decreased induction of genes linked to transcriptional regulation, growth, synaptic activity, and neuroprotection. Loss of E2 responsiveness is associated with a decrease in the expression of estrogen receptors, estrogen receptor α (ERα) and estrogen receptor β. Indeed, differential expression of estrogen receptors across hippocampal regions, and a decline in expression during aging suggest that receptor expression may contribute to differences in responsiveness and vulnerability to cellular stress. While the expression profile of the estrogen receptor is well characterized by age and brain region, the molecular mechanisms that regulate expression of estrogen receptor proteins are not well understood. One possibility is an epigenetic regulation of mRNA through methylation of cytosines in guanine-cytosine-rich areas of the gene promoter region, termed CpG islands. In this study, we examined ERα mRNA in hippocampal regions CA3 and CA1 in young (4 months) and aged (19 months) Fischer 344 ovariectomized rats. Furthermore, in order to delineate the potential epigenetic mechanism for transcriptional regulation of the ERα, we employed bisulfite sequencing to quantify DNA methylation of the 17 CpG sites in the exon 1b region of the ERα promoter. The results indicated that ERα transcription was higher in the CA3 region in young ($p< 0.001$) and aged ($p< 0.005$) rats. In addition to the regional differences, there was an age-related increase in the levels of ERα mRNA in the CA1 area of aged animals ($p< 0.05$) and a tendency ($p= 0.09$) for increased expression in the CA3 region of aged animals. Examination of promoter methylation suggested that the age difference in mRNA in the CA1 region may be related to higher methylation ($p< 0.05$) in the young rats. In particular, sites 1, 11, and 17 in CA1 exhibited higher levels of methylation in young animals, indicating that methylation at these specific sites may contribute to the regulation of ERα transcription in the hippocampus. Interestingly, a *post hoc* analysis of the methylation in the CA3 area showed that site 17 was highly methylated in young compared to aged animals. Thus, methylation of site 17 may contribute to the tendency for increased ERα mRNA in the CA3 region of aged animals, and suggests that additional methylation of sites 1 and 11 may be needed to enhance this epigenetic regulation. Together, these results suggest that differential methylation of the ERα promoter, specifically at sites, 1, 11, and 17, may explain transcriptional differences observed across age groups.

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Poster

758. Sex and Steroids

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Support: NIH/NIA Grant R01 AG010606

NIH/NIA Grant P01 AG16765

Intramural Research Program of the NIA

Title: Synaptic distribution of GPR30 in the aged monkey prefrontal cortex is associated with the procognitive effects of estrogen replacement therapy

Authors: *J. L. CRIMINS[1], C.-J. WANG[2], F. YUK[1], R. PURI[1], W. G. JANSSEN[1], Y. HARA[1], P. R. RAPP[3], J. H. MORRISON[1];
[1]Neurosciences, Mount Sinai Sch. of Med., New York, NY; [2]Univ. of Colorado Sch. of Med., Aurora, CO; [3]Natl. Inst. on Aging, Baltimore, MD

Abstract: Age- and menopause-related impairment in higher-level cognitive functions, such as working memory mediated by the dorsolateral prefrontal cortex (dlPFC), occurs in both humans and nonhuman primates. In aged rhesus monkeys that have undergone surgical menopause (ovariectomy), long-term cyclic administration of 17beta-estradiol (E2) reverses these cognitive deficits. However, the precise mechanisms by which estrogen exerts its procognitive effects are currently unknown. The estrogen-sensitive G-protein-coupled estrogen receptor 30 (GPR30; also known as GPER) is expressed in multiple regions of the mammalian brain including the forebrain, and has been identified specifically within the postsynaptic density (PSD) of rat hippocampal dendritic spines. Recent studies suggest that GPR30 modulates synaptic plasticity through rapid, non-genomic signaling and that its activation may have neuroprotective effects in ovariectomized animals. In the present study, aged vehicle- and estrogen-treated ovariectomized female rhesus monkeys were first tested on a battery of cognitive tasks that included a delayed response (DR) test of spatial working memory. Then, quantitative electron microscopic immunocytochemistry was used to examine the effects of estrogen treatment on the subcellular distribution of GPR30 in layer III dlPFC, including possible correlates with cognitive performance. Preliminary data indicated that regardless of treatment group, the majority (~84%) of dlPFC spines contained GPR30, which was predominately localized to synaptic, cytoplasmic, and plasmalemmal domains. In addition, GPR30-labeled spines were significantly larger than unlabeled spines and many of these possessed a large perforated PSD (~54% of labeled spines), a

synaptic subclass implicated in plasticity. A strong positive correlation emerged between GPR30-containing perforated synapse size, as measured by maximum PSD length, and working memory performance (average DR accuracy). Intriguingly, this relationship was maintained when the analysis was confined to the estrogen-treated group. Taken together, these findings suggest that GPR30 may be preferentially localized to distinct domains within a specific spine subclass and is strategically positioned to play an estrogen-dependent role in preserving the integrity of working memory with age and menopause.

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Poster

758. Sex and Steroids

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Topic: F.04. Neuroethology

Support: RR280112 (W.P)

IN200512 (R.P)

152872 (W.P)

167101 (R.P)

Title: Effects of mating and sexully relevant odorants in the arrival of new cells in the olfactory bulb of male mice

Authors: M. A. VELAZCO-MENDOZA, *W. PORTILLO, R. G. PAREDES; NEUROBIOLOGIA CONDUCTUAL Y COGNITIVA, INSTITUTO DE NEUROBIOLOGIA, QUERETARO, Mexico

Abstract: In the adult mammalian brain, there is a constant process of generation and incorporation of new cells throughout the life of the animal. The olfactory bulb (OB) is one of the structures where neurogenesis (generation of new neurons) is observed in the adult. This process relies in part on external stimulation. For example, previous studies have provided evidence that exposure to an enriched odor environment, to female pheromones and mating stimulation increases neurogenesis in the OB in adult rodents. The aim of the present study was

to evaluate if sexual behavior increases the number of new cells that arrive to the main and accessory olfactory bulb (MOB and AOB respectively). For this experiment we used C57BL/6J males mice divided into three groups of mice: controls, exposition and ejaculation. Control males were left alone in their home cage, exposition males were exposed to a sexually receptive female mouse but they did not have any sexual contact and ejaculation males had sexual behavior until one ejaculation. In the day of the behavioral test, males were given three injections of the DNA marker 5-bromo-2-deoxyuridine (BrdU, 100 mg/kg), the first one was two hours before the test, the second was immediately after the test and the third injection was two hours after the test. Fifteen days after the test, males were anesthetized with sodium pentobarbital and perfused, the brains were removed and sliced in sagittal sections. Slices were process for inmunohistochemestry to visualize BrdU positive cells in the granular, mitral and glomeural cell layers of the MOB and AOB. Preliminary data show not significant differences between groups in the number of new cells in any of the layer analyzed. Thus, sexual behavior and stimulation with sexually relevant odors did not increase neurogenesis in the OB.

Disclosures: **M.A. Velazco-Mendoza:** None. **W. Portillo:** None. **R.G. Paredes:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Topic: G.04. Physiological Methods

Support: JSPS KAKENHI Grant Number 25890024

Title: Two-choice behavior on different rules in head-fixed mice

Authors: ***K. TAO**, S. FUJISAWA;
Lab. for Systems Neurophysiol., RIKEN Brain Sci. Inst., Wako, Saitama, Japan

Abstract: Advances in genetic manipulation techniques have established the mouse as a prominent model system to investigate the neural circuits underlying behavior. In conjunction with electrophysiological recording, recent optogenetic methods can provide an unequivocal access to neuronal activity of a specific cell type. However, the complex head-mount apparatus can severely hinder voluntary movement, especially when recording from multiple brain regions. Here, we therefore introduce a behavioral method for head-fixed mice. Under the water-restricted condition, head-fixed mice received auditory stimuli and were required to lick either

one of two optical lickports which were positioned in front of their mouth to get a drop of water as a reward. Mice could perform this simple discrimination task hundreds of trials per behavioral session with high accuracy up to 95%. By developing this scheme, we further have established several cognitive behavioral paradigms which are thought to require prefrontal activity, such as extra-dimensional set-shifting task and spontaneous alternation task. While mice were performing the behavioral task, we successfully recorded neuronal activity from multiple brain regions such as dorsomedial prefrontal cortex and hippocampus in combination with optogenetic manipulation. These behavioral paradigms for head-fixed mice enable a variety of behavioral tasks, facilitating the system-level analysis of higher order behavior in mice. In principle, this procedure can be extended by including multimodal stimuli, such as visual or olfactory cues.

Disclosures: K. Tao: None. **S. Fujisawa:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Topic: G.04. Physiological Methods

Support: NSERC

Title: a novel drug-screening platform uncovers vorinostat as a potential anti-epileptic drug

Authors: *K. IBHAZEHIEBO[1], C. GAVRILOVICI[2], S. NAKANISHI[2], J. M. RHO[2], D. M. KURRASCH[1];
[1]Med. genetics, [2]Peadiatrics, Univ. of Calgary, Calgary, AB, Canada

Abstract: Epilepsy is a common neurological condition that affects approximately 1-2% of the general population. 30-40% of patients are unresponsive to drugs and continue to have unremitting recurrent seizures and attendant life-long cognitive, behavioral and mental health problems. Here, we generated epileptic zebrafish model to use in a screen for novel anticonvulsant drugs. Specifically, we introduced mutations into the zebrafish ortholog of *KV1.1* human gene (*kcna1*) associated with epilepsy. We validated our mutant using a combination of commonly accepted behavioral and cellular electrophysiological measures. A library of 143 compounds were screened through both our *kcna1* mutant and the commonly used pentylentetrazole (PTZ) model, which induces seizure pharmacologically. High throughput behavior assay that monitors locomotion tracking was used to screen all compounds.

Specifically, swim activity at baseline and following addition of compounds were analyzed in both models using the 96-well Zebrabox System (Viewpoint, France). High-velocity movements (>20 mm/s) corresponded to paroxysmal seizure-like convulsion. Drugs that blocked high-velocity swim movements in our each model by >40% were deemed candidates. Interestingly, despite increased locomotor activity in all both models - PTZ-induced and *kcna1* mutants - 9 of the 143 compounds were efficacious in *kcna1* mutants, whereas 33 of the 143 compounds were efficacious in PTZ-induced model, and only 2 compounds were efficacious in both. Vorinostat (HDAC-inhibitor) was our top hit in both models. Next, we examined the effect of Vorinostat on electrical activities in *kcna1* zebrafish brains using electroencephalogram (EEG) recording, and observed that vorinostat effectively reduced the frequency and duration of seizure-like activities to WT levels. Currently, we are examining the effects of vorinostat on brain electrical activities in *Kv1.1* mutant rodent models using video EEG recordings. Our approach to drug screening represents a new direction that could be used to identify novel therapeutics for monogenic intractable epilepsy disorder.

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Poster

759. Novel Assays: Nanotools for Neuroscience II

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Topic: G.04. Physiological Methods

Support: NanoQuebec

Title: Microfluidic devices to artificially connect neurons

Authors: *M. MAGDESIAN[1], M. LOPEZ[1], M. MORI[1], D. OLIVER[1], W. PAUL[1], Y. MIYAHARA[1], D. BOUDREAU[2], Y. DE KONINCK[2], P. GRÜTTER[1];
[1]Physics, McGill Univ., Montreal, QC, Canada; [2]Inst. Universitaire en Santé Mentale de Québec, Univ. Laval, Quebec, QC, Canada

Abstract: There are several limitations to study the mammalian nervous system. Difficult access and very small dimensions are recurring problems in neuroscience. Neurons extend through different organs creating a very complex network, where each connection has a specific role, fundamental to the proper function of the whole system. Neurons communicate through

synapses, which vary in size in the nanometer range. To better understand neuronal function, degeneration and repair, smaller and more precise tools are needed. Our work is the result of an interdisciplinary collaboration between cell biologists, physicist and engineers to develop new micro and nano tools for biological applications. We used microfluidics and silicone based microdevices to replicate the cell natural environment *in vitro*, improving neuronal culture models. Our microdevices enable complete control of the cell position and microenvironment, providing important insights into neuronal growth and degeneration. In the present work we use rat hippocampal neurons grown in microfluidic devices, combined with Atomic Force Microscopy (AFM) and optical microscopy to manipulate axonal growth, synapse formation and connectivity. We attached functionalized beads to the tip of an AFM cantilever are precisely positioned a bead on top of one axon. Once the beads adhere to the axon, we use the AFM to pull the beads, thereby mechanically inducing the growth of a new neurite for several hundreds of microns. When in contact with another neuron the newly formed neurite form a stable connection. To test the functionality and the type of connection, we put together an electrophysiology setup which enables paired recordings. We faced several instrumental and biological challenges to keep neurons healthy and responsive to perform paired recordings after the formation of the artificial synapse. From connection to recording, we developed different devices that facilitate the study of neuronal development and degeneration. Now we have instrumentation reliability allowing experiments to be carried out routinely and we will discuss potential biological factors that may lead to the creation of strong, mature and stable artificial connections.

Disclosures: **M. Magdesian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); McGill University. **M. Lopez:** None. **M. Mori:** None. **D. Oliver:** None. **W. Paul:** None. **Y. Miyahara:** None. **D. Boudreau:** None. **Y. De Koninck:** None. **P. Grütter:** None.

Poster

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Title: A method for optical measurement of footfalls in head-fixed, awake behaving mice with simultaneous electrophysiological recordings in sensorimotor cortex

Authors: *J. B. SMITH[1], D. SHRIVER[2], P. J. DREW[1,3];
[1]Ctr. For Neural Engin., [2]Dept. of Mechanical Engin., Pennsylvania State Univ., University Park, PA; [3]Dept. of Neurosurg., Penn State Col. of Med., Hershey, PA

Abstract: Locomotion is an innate sensorimotor behavior that does not require training and is a valuable assay for evaluating deficits in neurological function. Spherical balls or treadmills are commonly employed for recording and imaging studies in which head-fixed mice are free to locomote, but these designs make it difficult to identify the precise timing of foot-placements. We are interested in studying the sensory evoked responses to foot contact in the forelimb/hindlimb representations in cortex. In order to detect footfalls during locomotion, we have built a transparent turntable with frustrated Total Internally Reflected (TT-fTIR) infrared light. When the paw of the animal contacts the TT-fTIR, the internally reflected light is deflected downward, out of the turntable. The contact of the paws with the TT-fTIR are visualized with a high-speed camera (up to 100 frames per second), and a semi-automated algorithm is used to detect footfalls of each of the paws from the video data. Locomotion speed is monitored with a rotary velocity encoder. The TT-fTIR enables us to investigate somatosensory and motor related neural activity correlated to velocity, as well as the timing of footfalls during voluntary running and walking. Recordings from the forepaw/hindpaw sensorimotor cortex with implanted stereotrodes and high impedance electrodes have showed that we can simultaneously record both running/footfall activity concurrently with neural activity. During locomotion, we observed a robust increase in gamma-band power in the local field potential (LFP), and an increase in the multi-unit (MUA) firing rate, relative to rest, in the somatosensory cortex. We have been able to create peri-footfall triggered averages of the LFP envelope and the MUA response to quantitatively assess foot contact induced modulation of cortical activity. Because our TT-fTIR uses infrared light to monitor footfalls in a head-fixed preparation, it is compatible with 2-photon microscopy, making it a useful tool for studying the neural circuits involved in locomotion.

Disclosures: J.B. Smith: None. **D. Shriver:** None. **P.J. Drew:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 759.05/VV59

Topic: G.04. Physiological Methods

Support: Bill & Melinda Gates Foundation

John Simon Guggenheim Foundation

Oregon Nanoscience and Microtechnologies Institute

NIAID Grant RAI087059Z

Title: Microfluidic devices for rapid quantification of pharyngeal activity by electrophysiological measures in nematode models of human diseases and parasitic nematodes

Authors: *S. R. LOCKERY[1,2], K. J. ROBINSON[1,2], W. M. ROBERTS[2], J. J. VERMEIRE[3], J. C. WEEKS[1,2];
[1]Inst. Neurosci, Univ. of Oregon, EUGENE, OR; [2]NemaMetrix, Eugene, OR; [3]UC San Francisco, San Francisco, CA

Abstract: There is growing interest in utilizing alterations in the pumping behavior of the nematode pharynx in screens of the biological activity of a wide variety of compounds. Applications include the search for novel anthelmintics, compounds that prolong health span, and potential drugs to ameliorate the effects of human neuro- and neuromuscular diseases, for which Caenorhabditis elegans is a validated model. In such investigations, pharyngeal pumping is currently measured by slow-motion replay of brief video recordings of feeding worms, during which pumps are counted by a human observer. Because this method is manual, the observation time for individual worms and the number of worms that can be processed per day are severely limited, as is the temporal resolution of pumping behavior. Another limitation is that weak pumps, such as those produced by drugged, aged or diseased worms, can be hard to detect reliably and accurately. Progress in pharynx-based screening would be greatly accelerated by longer observation times and the ability to count pumps automatically at high temporal resolution. To address this issue, we have developed two broad classes of microfluidic devices (elastomeric "chips") that record electropharyngeograms, the electrical signals emitted by the pharynx, thereby enabling automated quantification of pharyngeal activity with millisecond temporal resolution. In one class of devices, eight or more nematodes can be recorded in parallel. The 8-channel device can be used after chronic exposure to test compounds, or before, during and after acute exposure to them. This platform has been validated in *C. elegans* and parasitic nematode species (e.g., Haemonchus contortus and Ancylostoma ceylanicum). Throughput can be increased by increasing the number of channels per chip or running multiple chips at once. In another class of devices, a population of tens to hundreds of worms can be loaded into a reservoir and individual worms can be positioned semi-automatically into an electrical recording module.

This type of device is particularly well suited to studies involving chronic compound exposure when large numbers of worms must be processed. In parallel, we are developing an inexpensive, stand-alone system that will allow researchers unfamiliar with electrophysiology to record and analyze pumping behavior automatically in hundreds of worms per day. In addition to accelerating research already in progress, this technology is likely to expand the range and complexity of biological problems that can be addressed by recording pharyngeal activity in a variety of nematode species.

Disclosures: **S.R. Lockery:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NemaMetrix, LLC. **K.J. Robinson:** A. Employment/Salary (full or part-time):; NemaMetrix. **W.M. Roberts:** A. Employment/Salary (full or part-time):; NemaMetrix. **J.J. Vermeire:** None. **J.C. Weeks:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NemaMetrix.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 759.06/VV60

Topic: G.04. Physiological Methods

Title: Bpod: An open source platform for precise behavioral measurement and control

Authors: *J. SANDERS, A. KEPECS;
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Abstract: Neural recordings during awake behavior are most interpretable with respect to precise behavioral and environmental measurements. Ideally, these measurements would be captured with sub-millisecond precision - the temporal precision relevant for alignment to single action potentials. However, implementing such precise instrumentation can be technically challenging, and prohibitively expensive when scaled to dozens of behavioral test rigs. To overcome these limitations, we developed Bpod - an open source platform for behavioral measurement and control, that costs ~$180 to build. Bpod's hardware expands on Arduino, an open embedded microcontroller platform, by providing modular interfaces to infrared photo-gates commonly used for decision capture in rodent research, solenoid valves for dispensing liquid reward or air puffs, logic lines provided as isolated BNC channels and a shift register for automatic event synchronization with electrophysiology systems. To encapsulate future hardware

extension, two 5V serial ports on Bpod's main module are paired with a custom-designed Arduino "Shield", for low-latency byte-code control of Arduino slave devices. One such slave device is demonstrated - an embedded sound server that plays any of 255 separate 44.1kHz stereo sounds on demand, with ~8ms latency and ~1ms jitter. For the protocol developer, each trial is programmed as a finite state matrix relating captured events to hardware states, which is then executed by Bpod in real-time. We demonstrate Bpod's precision and reliability, its MATLAB protocol development tool suite, and examples of behavioral and neural data captured with the system. We anticipate that with its flexibility and low cost, Bpod will be a useful starting point for labs seeking to implement precisely controlled rodent behavior.

Disclosures: J. Sanders: None. **A. Kepecs:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 759.07/VV61

Topic: G.04. Physiological Methods

Title: A new method for determining the degree of sedation in beagle dogs; validation with propofol and comparison to a novel compound

Authors: *G. M. BELFORT[1], R. S. HAMMOND[1], W. W. MUIR[2], Y. UEYAMA[2], S. J. KANES[1], G. M. BOTELLA[1], F. G. SALITURO[1], A. J. ROBICHAUD[1], J. J. DOHERTY[1]; [1]Sage Therapeut., Cambridge, MA; [2]QTest Labs, Columbus, OH

Abstract: An important obstacle to developing novel drugs targeting specific levels of sedation (e.g., moderate sedation) is the paucity of sensitive preclinical assays. Loss of righting reflex in rodents and the induction of lateral recumbency in dogs are useful as gross measures of sedation, but not for measuring the degree of sedation. Here we report a new behavioral method for measuring multiple levels of sedation in beagle dogs. This method is comprised of a combination of auditory and somatosensory stimuli of increasing intensity to define four levels of alertness/sedation: 1) Awake, 2) Moderate Sedation, 3) Deep Sedation and 4) General Anesthesia. Validation studies were performed with propofol and involved the rapid induction of general anesthesia (6 mg/kg IV over 1 minute) followed by sequential decrementing constant rate infusion steps (range: 400 - 105 µg/kg/min), each lasting 50 minutes. Behavioral testing occurred after the bolus administration and at the 45 minute time point of each constant rate infusion step. Test article plasma concentration was measured 3 minutes after the bolus and at 15

minute intervals throughout the study. In addition, cardiorespiratory monitoring was performed on all animals throughout the study. When plasma concentrations of propofol were normalized to the highest value observed during normal alertness (awake), small increases were required to achieve moderate sedation (MS, 1.6-fold), deep sedation (DS, 1.8-fold), and general anesthesia (GA, 2.5-fold). Furthermore, the animals developed hypoxemia ($PaO_2 < 60$ mmHg) at an average plasma propofol concentration only 3-fold higher than the level required to achieve GA. In contrast, the novel synthetic neuroactive steroid (NAS) sedative, SGE-746, exhibited a more gradual exposure-response relationship than propofol. When normalized to the test article plasma concentration upon recovery from sedation (awake), much larger increases were required to achieve moderate sedation, deep sedation, and general anesthesia with SGE-746 (MS: 4.2-fold, DS: 5.3-fold, GA: 7.3-fold) relative to propofol. Cardiorespiratory stopping criteria were not encountered with SGE-746, in part due to limits on the volume that could be safely administered. Results of these studies recapitulate the clinical observation that small increases in propofol plasma concentration result in a rapid transition through multiple depths of sedation to respiratory compromise.

Disclosures: **G.M. Belfort:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **R.S. Hammond:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **S.J. Kanes:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **G.M. Botella:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **F.G. Salituro:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **A.J. Robichaud:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **W.W. Muir:** 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.; QTest Labs. **Y. Ueyama:** 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.; QTest Labs.

Poster

759. Novel Assays: Nanotools for Neuroscience II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 759.08/VV62

Topic: G.04. Physiological Methods

Title: Dual tracer receptor occupancy for 5-HT_{1A} and SERT using non-radiolabelled tracers in rats

Authors: J. THENTU, *G. BHYRAPUNENI, D. AJJALA, R. ALETI, S. SRIKAKOLAPU, R. NIROGI;
Discovery Res., Suven Life Sci., Hyderabad, India

Abstract: Measuring the degree to which the test drug occupies its receptors play a significant role in advancing the compounds in CNS drug discovery. If the compound has affinity towards multiple receptors, measuring in-vivo occupancy simultaneously will be of immense interest. Utility of multiple non-radiolabelled tracers to measure the distribution of tracers for occupancy measurement in a single animal was accomplished using LC-MS/MS mode of analysis. Current focus on antidepressant research involves targeting multiple receptors or transporters in order to achieve quicker onset of action and minimal side- effects thus monitoring receptors occupancy simultaneously becomes important. In this report, we developed simultaneous in-vivo receptor occupancy assay for 5-HT_{1A} and SERT using two different non-radiolabelled tracers, WAY 100635 and DASB, respectively at 3 and 10 µg/kg dose. The tracers used in this study were chosen based on information from the literature. Individual assays for each target were set and then a "dual tracer" assay was established where both tracers were administered simultaneously. Assay was validated with various doses of pindolol (0.1 to 10 mg/kg, *i.v*) and paroxetine (0.03 to 3 mg/kg, *i.p*) in rats. The ED_{50} vales were similar when tested alone or in dual tracer assay. This is the first reported method to simultaneous determine the occupancy of both receptors in the same animal. The method can be employed in screening the novel compounds at lead optimization phase.

Disclosures: **J. Thentu:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **D. Ajjala:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **R. Aleti:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **S. Srikakolapu:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.,Hyderabad, India.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Program#/Poster#: 759.09/VV63

Topic: G.04. Physiological Methods

Support: R21 NS087479

Title: A portable low cost multifunction electrophysiology system developed on open source hardware

Authors: *X. LI, K. BORGES, G. SHEPHERD;
physiology, Northwestern Univ., Chicago, IL

Abstract: In order to investigate the functional neural circuit related to mouse motor system, we developed a novel, portable and low cost behavior training, monitoring, stimulating and neural recording system. All the electronic modular devices were developed on open source hardware. In the behavior paradigm, the mouse's locomotion which drove the trackball or sphere tread mill was interfaced with an Arduino microcontroller programmed with the task paradigm. A Raspberry Pi or BeagleBone Black single board computer as the host controller, set the parameter of the behavior paradigm, collected, stored and visualized the mouse behavioral data streamed from this "behavior Arduino". For the closed-loop experiment, another "stimulation Arduino" was programmed as the stimulation controller for electrical or optogenetic stimulation and was also hosted by the host controller. In the neural recording system, Intan multichannel amplifier combined with Opal Kelly FPGA-USB module board was adopted. The recorded electrophysiological data were streamed to a laptop or desktop computer depending on the need of portability. The programming for the Arduino was in C++ like IDE. Due to the Linux platform in Raspberry Pi and BeagleBone Black, the software was written in Python which is multiplatform compatible. The neural data acquisition and online analysis software was developed in C++, Matlab or Python could be used for offline data analysis.

Disclosures: **X. Li:** None. **K. Borges:** None. **G. Shepherd:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Program#/Poster#: 759.10/VV64

Topic: G.04. Physiological Methods

Support: NIH Intramural Funding

Title: An open source operant conditioning chamber

Authors: *K. DEVARAKONDA, A. V. KRAVITZ;
NIDDK, Bethesda, MD

Abstract: The operant conditioning chamber is a cornerstone of animal behavioral research. Operant boxes test learning and motivation in animals, particularly for food reward. However, commercial operant chambers can cost several thousands of dollars. We have constructed an inexpensive and easily assembled open-source operant chamber based on the Arduino microcontroller platform that can be used to train mice to respond for a reward. The apparatus contains two nose pokes, a drinking well, and a solenoid-controlled sucrose delivery system. The chamber can easily run fixed ratio and progressive ratio training schedules, and can be programmed to run more complicated behavioral paradigms. Additional features, such as coordinate and video tracking, can be easily added to the operant chamber through the array of widely available Arduino-compatible sensors. The chamber's design files and programming code are open source and available online for others to use.

Disclosures: K. Devarakonda: None. **A.V. Kravitz:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Program#/Poster#: 759.11/VV65

Topic: G.04. Physiological Methods

Support: NIH RC4 073008

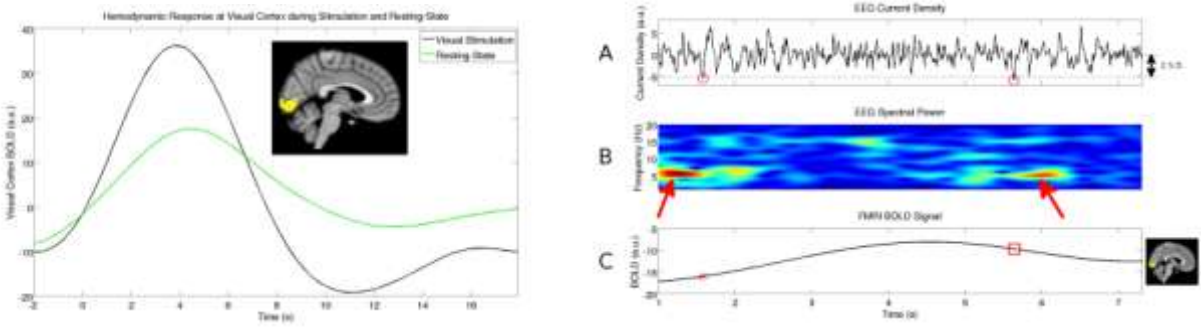
5 T32 AG 25814

Title: Estimating the hemodynamic response to neural activation in resting-state fMRI

Authors: *P. BOORD, T. J. GRABOWSKI, Jr;
Radiology, Univ. of Washington, Seattle, WA

Abstract: We have developed a non-invasive method to estimate the hemodynamic response (HR) to neural activation in human subjects at rest. The method uses EEG simultaneously measured with fMRI during the resting-state. Current (neural) source density (CSD) is determined at the scalp by calculating the surface Laplacian of the EEG. Neural activation events are designated as the largest CSD spike exceeding a threshold within contiguous windows. The HR is then obtained by either averaging the BOLD signal time-locked to each spike, or

deconvolving the BOLD signal with the spike sequence. We collected 10 minutes of simultaneous EEG-fMRI data in a subject at rest, and 20 minutes of data (4 repeats of 5 minutes of fMRI) while the subject performed a visual stimulus/response task. The task involved the subject pressing buttons in both hands using their thumbs in response to a flashing checkerboard presented at 20-second intervals. Simultaneous EEG-FMRI was recorded using a Brain Products 64-channel BrainAmp MR in-magnet EEG amplifier, and a BrainCap MR EEG electrode cap. We successfully removed the MRI gradient and ballistocardiographic artifacts using the FMRIB plug-in for EEGLAB. Neural activation events were determined from spikes exceeding a threshold of two standard deviations within 20-second contiguous windows of EEG. The method will allow estimation of HR parameters throughout the cortex related to neurovascular coupling, and potentially provide a valuable clinical tool for the assessment of brain health.



Disclosures: **P. Boord:** None. **T.J. Grabowski:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Topic: G.04. Physiological Methods

Support: Funded by European Union and the Free State of Saxony (SAB 100121467)

Title: Freely vibrating nanostructured scaffolds as a novel assay to investigate mechanical properties of retinae

Authors: ***S. RAHMAN**[1,2,3], A. REICHENBACH[1], S. MAYR[2,4], M. ZINK[3];
[1]Paul Flechsig Institue for Brain Res., Leipzig, Germany; [2]Leibniz Inst. for Surface Modification, Leipzig, Germany; [3]Soft Matter Physics Div., Fac. of Physics and Earth Sciences,

Inst. of Exptl. Physics 1, Univ. of Leipzig, Leipzig, Germany; [4]Translational Ctr. for Regenerative Med., Univ. of Leipzig, Leipzig, Germany

Abstract: Mechanical properties of the adult mammalian retina play a crucial role in many eye diseases such as retinoschisis where the retina swells, Müller cells are overstretched, become mechanical instable and rupture. Studying these properties offers new perspectives for novel therapeutic approaches. However, measuring the elasticity of adult retina whole-mounts is difficult and hardly investigated until now. Recent studies use atomic force microscopy to probe local retinal tissue elasticity on the surface with a resolution of micrometers. Here we show a novel technique to study the global retinal mechanical properties in terms of elasticity and energy dissipation during mechanical probing. In a first step, titanium dioxide nanotube arrays are employed as scaffold to culture an adult guinea pig retina explant on. As we have shown recently, these scaffolds, which comprise parallel aligned nanometer-size tubes of TiO2, can be employed for long-term culture of adult neuronal tissue such as the retina [1]. The scaffolds are produced by an anodization process, while the tube diameter that determines the interaction of the scaffold with the tissue is controlled by varying the applied voltage. For our assay the retina is first cultured on the scaffold. Afterwards the scaffold acts as a freely vibrating reed, viz. the solid rectangular scaffold is clamped to a holder on one end and excited with a motor to oscillate freely. During vibration the frequency and the damping of the reed with the retina on top is detected by a laser. From the eigenfrequency of the vibrating reed before and after retina culture, the elastic properties of the retina, i.e. the Young's modulus can directly be calculated [2]. From the damping of the oscillation amplitude, dissipated energy by the tissue, which can result from internal structural changes, is extracted. Moreover, the change in vibration frequency of the empty reed with the reed with retina offers the possibility to measure the interaction of the retina with the scaffold. Since proper adhesion of tissue to the scaffold material is a prerequisite for long-term culture, our assays also probes this interaction quantitatively. Together with Finite Element Simulations to further validate the obtained results, we gain new insides into the global mechanical properties of the retina with a quantitative new analysis method. Since we have already demonstrated that TiO2 nanotube scaffolds are suitable for long-term culture of adult neuronal tissue of the brain as well, our new assay can directly be employed to study the mechanical properties of the brain. [1] Dallacasagrande et al., Adv. Mater. 24, 2399 (2012) [2] K. Fischer & S.G. Mayr, Adv. Mater. 23, 3838 (2011)

Disclosures: **S. Rahman:** None. **A. Reichenbach:** None. **S. Mayr:** None. **M. Zink:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Craig H. Nielsen Foundation

Title: Use of electric field sensors for continuous non-contact recordings of motor behaviors in rodents

Authors: *M. L. MCKINNON, D. NOBLE, C. J. MACDOWELL, T. I. NEBLETT, M. HALDER, W. N. GOOLSBY, S. M. GARRAWAY, S. HOCHMAN;
Emory Univ., Decatur, GA

Abstract: Behavioral neuroscience studies rely heavily upon the accurate quantification of animal behavior in the rodent. However, behavior can vary significantly depending on confounding factors in the laboratory environment including: (i) variability in stress exposure associated with transport/handling, (ii) surgical interventions, and (iii) the testing environment. Recording physiological and behavioral variables in a home-cage environment without experimenter intervention is desirable, but current systems for automated home-cage recording of behavioral and physiological variables are expensive and some require devices that are surgically implanted or chronically affixed to the animal. To assess behavioral and physiological measures non-invasively and without human interaction, we tested whether a newly available electric field sensing chip (EPIC chips; Plessey Semiconductors) could serve as an inexpensive, non-contact method to record respiratory rate and motor behaviors continuously in the home-cage. We tested these chips in the home-cages of Sprague-Dawley rats and C57BL6 mice. EPIC chips were affixed to the exterior of the cage or placed within tube shelters. Movement-related voltage changes were observed during periods of gross motor activity. Voltage oscillations of characteristic frequencies were also seen during more subtle repetitive motor behaviors such as stationary respiration (approximately 2 Hz in rats), grooming (3-6 Hz), and sniffing (7-8 Hz), as verified with simultaneous video recordings. Additional experiments utilizing the EPIC chips in a plethysmography chamber further confirmed accuracy in measured respiratory rate. We are now testing battery-powered dataloggers for use in continuous remote capture of rodent behavior in the vivarium home-cage environment for upwards of 24 hours. One goal is to better understand the contribution of inter-animal variability and transitions in measured behaviors to observed outcomes in experimental tests.

Disclosures: **M.L. McKinnon:** None. **D. Noble:** None. **C.J. MacDowell:** None. **T.I. Neblett:** None. **M. Halder:** None. **W.N. Goolsby:** None. **S.M. Garraway:** None. **S. Hochman:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Program#/Poster#: 759.14/VV68

Topic: G.04. Physiological Methods

Title: SERCaMP: A reporter to monitor endoplasmic reticulum calcium homeostasis in neurons

Authors: *K. A. TRYCHTA, E. M. SIMONS WIRES, C. T. RICHIE, B. K. HARVEY, M. J. HENDERSON;
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Abstract: Under normal conditions, the endoplasmic reticulum (ER) maintains a high concentration of calcium in the lumen, with levels 1000 to 10,000 times higher than in the cytoplasm. ER calcium is important for many cellular functions, such as protein folding, lipid metabolism, and signaling pathways. Disruption of ER calcium homeostasis is implicated in neurodegenerative diseases including Alzheimer's, Parkinson's, and Huntington's. However, examining progressive ER calcium dysregulation during disease pathogenesis is difficult due to the limitations of current technologies. Towards the goal of longitudinally monitoring ER calcium homeostasis, we have developed SERCaMPs, or secreted endoplasmic reticulum calcium monitoring proteins. SERCaMP trafficking is controlled by a carboxy-terminal amino acid sequence (ASARTDL) that localizes proteins to the ER and causes secretion that is dependent on ER calcium depletion. In this way, SERCaMPs can be used to monitor ER calcium perturbations in cells over time by repeatedly sampling extracellular fluids, like culture media, and measuring secreted SERCaMP levels. Gaussia luciferase (GLuc)-based SERCaMPs allowed for detection of ER calcium depletion in as few as 20 neuroblastoma cells. Adeno-associated viral (AAV) vectors encoding GLuc-SERCaMPs were used to study ER calcium homeostasis in rat primary cortical neurons. We observed ER calcium depletion in stress models including hyperthermia, glutamate toxicity, and paraquat exposure. SERCaMPs can also be used to monitor calcium homeostasis *in vivo* by sampling blood, and SERCaMP release from dopamine neurons into cerebrospinal fluid (CSF) is under investigation. Overall, SERCaMP technology provides a simple and sensitive approach to assess ER calcium in a variety of cell types, including neurons, and holds potential to further our understanding of the relationship between ER calcium and disease pathogenesis.

Disclosures: K.A. Trychta: None. **E.M. Simons Wires:** None. **C.T. Richie:** None. **B.K. Harvey:** None. **M.J. Henderson:** None.

Poster

759. Novel Assays: Nanotools for Neuroscience II

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Topic: G.04. Physiological Methods

Title: Human neural microtissues derived from induced pluripotent stem cells for toxicity testing

Authors: S. DELAURA[1], D. A. FLURI[2], R. MARCHAN[3], W. MORITZ[2], *E. M. JONES[4], J. G. HENGSTLER[3], J. M. KELM[2];
[1]Cell. Dynamics Intl., Madison, WI; [2]InSphero AG, Schlieren, Switzerland; [3]Leibniz Res. Ctr. for Working Envrn. and Human Factors, Dortmund, Germany; [4]Gist Consulting, MADISON, WI

Abstract: *In vivo* animal models are primarily employed to assess for neurotoxic effects of chemicals and potential candidate compounds in drug development. *In vitro* testing is mainly limited to HTS compatible low complexity 2D cultures using cell lines and more sophisticated low throughput explant rodent cultures. Cell sourcing for primary human brain material is inherently difficult and ethically controversial. Recently developed strategies to generate embryonic stem cell-/induced pluripotent stem cell- (ESC/iPSC) derived neuronal and glial cell types offer an invaluable source to create a new generation of *in vitro* test systems for neurotoxicity, drug discovery, and disease modeling. We report here the generation of a scaffold-free 3D microtissue model derived from human iPSC- derived cell types. The spheroidal aggregates are produced in high throughput compatible hanging drop plates. Owing to the standardized production process, microtissues are highly size consistent and culture handling, such as maintenance, compound dosing or downstream analytics are compatible with robotics. The human brain microtissues consist of iPSC-derived astrocytes in co-culture with iPSC-derived neurons. Microtissue constructs exhibit stable three-dimensional architecture over time periods longer than four weeks and display positive staining for the neuronal markers β-III-tubulin, synaptophysin and the astrocyte marker GFAP. These microtissue models provide three-dimensional biological complexity with high reproducibility. When combined with high throughput automation, this *in vitro* screening-friendly system closely recapitulates *in vivo* human biology enabling new choices for phenotypic screening.

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Poster

759. Novel Assays: Nanotools for Neuroscience II

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Program#/Poster#: 759.16/VV70

Topic: G.04. Physiological Methods

Title: Cisterna magna ported (CMP) conscious rhesus monkey model: An innovative approach to neuroscience - evaluation of CSF biomarkers for Alzheimer's disease

Authors: *M. S. MICHENER[1], D. GILBERTO[1], B. E. SMITH[1], C. JOHNSON[1], G. WU[2], J. KALININA[4], A. MCCAMPBELL[5], L. HANDT[1], M. SAVAGE[3], S. MOTZEL[1];
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Abstract: The CMP Model in non-human primates enables repeated, minimally invasive collection of cerebral spinal fluid (CSF) from conscious rhesus monkeys. Surgery allows for a flexible silicone catheter to be implanted into the cisterna magna above the C1 vertebrae. This catheter is attached to a titanium port positioned subcutaneously between the scapulae to allow CSF to flow when accessed. The system is gravity-driven; therefore, there is no need to apply negative pressure to the catheter and potentially damage brain tissue. The CMP monkey colony is used primarily to evaluate the potential of multi-mechanistic agents to alter CSF and plasma biomarkers of Alzheimer's disease (AD), with the ability to concurrently measure CSF and plasma drug concentrations. The AD brain is characterized neuropathologically by the presence of amyloid β-peptide (Aβ) containing plaques along with neurofibrillary tangles. Aβ generation depends on the proteolytic cleavage of the amyloid precursor protein (APP); which is a prime therapeutic target for the treatment of AD. In the NHP model CSF samples have been evaluated for diurnal, baseline or post drug levels of the following peptide or protein biomarkers associated with AD: CSF Aβ40, Aβ42, Aβ oligomers, APPα, sAPPβ, ApoE and Tau. Neurotransmitters implicated in cognition have also been studied in this model; including the cholinergic and glutamatergic antagonist kynurenic acid (KYNA), which when chronically elevated could impair cognition. CSF KYNA was evaluated, comparing young and older CMP monkey cohorts. This innovative and resource-sparing model is a powerful research tool which has accelerated the development of numerous novel central nervous system therapeutics from the pre-clinical to clinical biological space.

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Poster

760. Network Models and Cognitive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 760.01/VV71

Topic: G.06. Computation, Modeling, and Simulation

Title: Exploring the role of hub structures in the changing properties of functional networks

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Abstract: The structural and functional connectivity of the brain forms complex networks that can be characterized using classic graph theory and informational analysis. While our understanding of that connectivity is incomplete, previous research has suggested that these networks display small-world (sw) properties, a pattern of connections that optimizes information flow (Watts & Strogatz, 1998, Sporns & Honey, 2006). These sw properties of functional networks and the flow of information appear to break down during unconscious states (Uehara et al., 2013, Ferrarelli et at., 2010). To explore the breakdown of these networks, a model was created to alter the structure of single as well as clustered sw graphs by applying two types of algorithms: a random algorithm that selects and moves edges in the network randomly, and several hub algorithms that move edges by first attacking the edges between hubs, and then the edges between hub and non-hub nodes. Each hub algorithm uses a different method to define hub nodes: eigenvector centrality, betweenness centrality, and node degree. Equivalent graphs were generated using the igraph Strogatz-Watz generator for a range of graph sizes. For clustered sw graphs, edges were generated between groups of single sw graphs. Next, the algorithms were applied, producing altered graphs for which various properties then were measured. Multiple seeds were run for each model to assess consistency. The results suggest that hubs as defined by different algorithms are different in terms of their role in global network properties. The hub definitions used do not always define hubs that are involved in maintaining or breaking down sw properties. In addition, clustered sw networks generate results that are more inline with expectation about sw property breakdown. As research on functional brain networks moves forward, locating and defining the different types of hubs within a network will help provide

insight into the role each may play, as well as how much control that region can influence on the global functional network.

Disclosures: R. Himmelwright: None. **E.R. Reynolds:** None.

Poster

760. Network Models and Cognitive Function

Location: Halls A-C

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Program#/Poster#: 760.02/VV72

Topic: G.06. Computation, Modeling, and Simulation

Support: Israel Science Foundation

Title: Global disconnectivity and loss of symmetry in mice knockout for the neurodevelopmental gene Ahi1

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Abstract: Introduction: The Abelson helper integration site 1 (AHI1) gene plays a pivotal role in brain development. Studies by our group and others have demonstrated association of AHI1 with schizophrenia and autism. Recently, we have shown that Ahi1 heterozygous knockout (Ahi1+/-) mice displayed an anxiolytic-like phenotype across different converging modalities. Using behavioral paradigms that involve exposure to environmental and social stress, significantly decreased anxiety was evident in the open field, elevated plus maze and dark light box, as well as during social interaction in pairs. Assessment of core temperature and corticosterone secretion revealed a significantly blunted response of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis in Ahi1+/- mice exposed to environmental and visceral stress. The current project extends previous findings by seeking a mechanistic explanation for the anxiolytic-like phenotype by using functional imaging and analysis of network organization. Methods: Mk-801 hyperlocomotion and fMRI were performed following Lotan et al (2014). Comparison of regional and global topological properties between networks was conducted using the Graph-Analysis Toolbox. Results: The Ahi1+\- mouse displayed an anxiolytic-like behavioral phenotype with exquisite sensitivity to NMDA-receptor blockade. Functional corticolimbic connections were sparse and asymmetrical in Ahi1+/- mice. An abnormal network organization and amygdalar disconnectivity were also noted in the mutant mouse. Conclusions: Taken

together, the current data suggest that aberrant Ahi1 expression could lead functional disconnectivity and abnormal network organization. This perturbations may, in turn, result in various cognitive-emotional phenotypes. Future studies will determine whether these findings arise from impaired ciliary function or from other ciliary independent mechanisms.

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Poster

760. Network Models and Cognitive Function

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 760.03/VV73

Topic: G.06. Computation, Modeling, and Simulation

Title: Differential correlation analysis as a new technique for probing interaction strength between brain areas when performing emotionally motivated willed motor activity

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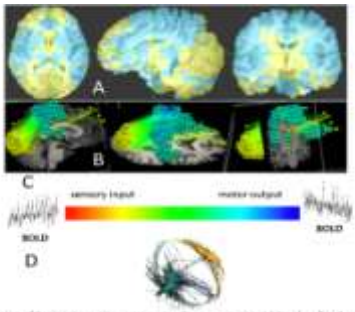
Abstract: Background: Quantification of interaction of sensory (input) and motor (output) brain regions may give insight into neural correlates of motor activity evoked by a sensory input. **Methods**: Functional magnetic resonance images (fMRI) (TR=1300ms) were obtained during the performance an approach-avoidance paradigm: A total of 10 faces (10 sec, interspersed by green background, 60 sec) were presented to 9 healthy subjects (6 male, average age: 34.7) who were instructed to squeeze a ball with their right hand if a face was perceived as unpleasant. Using differential correlation analysis (DCA) a difference correlation map was calculated from the BOLD signal time course of the primary visual cortex (sensory input) and of the motor cortex (motor output) (figure 1a). Networks using a 2D spring-embedded design and using the 3D anatomical Talairach space with correlation coefficients of signal time courses as edge weights were created (figure 1b and c). **Results**: On average, visual cortex, cuneus, precuneus, dorsal lateral prefrontal cortex, cingulate gyrus, parahippocampal gyrus, thalamus, lentiform nucleus, caudate and substantia nigra exhibited stronger correlation with the sensory (input) time course, while postcentral gyrus, supplementary motor area, precentral gyrus, insula and regions in the cerebellum showed stronger correlation with the motor (output) time course. **Conclusion:** Differential correlation analysis in combination with a graph-theoretical approach revealed

interaction strengths of the sensory and motor brain networks activated during the performance of a willed motor activity initiated by an emotional stimulus.



A: Division of brain into regions processing the sensory input (visual, yellow) and the motor output obtained through differential correlation analysis (DCA).
B: Visualization of interactions of brain regions by 3D graph display overlaid onto anatomical images. Colors correspond to color scale in C, with red/yellow colors indicating sensory processing and green/blue colors denoting motor output.
C: On left: BOLD time course of visual cortex. Red stars mark appearances of faces corresponding to peaks in signal. On right: BOLD time course of motor cortex. Red stars mark fist squeezing corresponding to peaks in signal.
D: Two dimensional graph layout of 3D graph shown in B. Red color marks voxels activated while processing sensory input, blue color marks voxels for motor output.

Disclosures: C. Karmonik: None. **A. Verma:** None. **S.H. Fung:** None. **R.G. Grossman:** None.

Poster

760. Network Models and Cognitive Function

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIBIB 2R01EB000840

COBRE 5P20RR021938/P20GM103472

Title: Meta-state analysis reveals reduced resting fMRI connectivity dynamism in schizophrenia, with dynamic fluidity and range further suppressed by many individual symptoms

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CA; [7]Dept. of Psychiatry, Univ. of Iowa Col. of Med., Iowa City, IA; [8]Dept. of Psychiatry, Duke Univ. Sch. of Med., Durham, NC

Abstract: Background: Network connectivity remains a central focus of much resting-state fMRI research. Until very recently, most studies have assumed functional network connectivity (FNC) to be effectively stationary in the resting brain. Interest is growing however in the dynamical properties of network connectivity. Our approach models FNC matrices computed on successive windowed segments of network timecourses (wFNCs) as weighted sums of maximally temporally independent *basis connectivity patterns* (BCPs). This approach is motivated by a desire to understand network connectivity dynamics in terms of (not necessarily observable) correlation patterns that "pipe in" and fade out of observed time-varying FNCs in a relatively independent manner. **Methods**: This study was conducted on a large fMRI dataset (N=314; 163 healthy (HC), 151 schizophrenia patients (SZ)). Data was preprocessed and decomposed into functional networks using group ICA. wFNCs were computed from subject-specific network timecourses. Five maximally mutually independent timecourses (TCs) with associated BCPs were then estimated from the wFNCs using group temporal ICA (tICA). Estimated TCs were discretized into signed quartiles (reassigned values in {±1,±2,±3,±4} designating quartile position among same-sign TC values) **Results**: We focus here on the general dynamic behavior of so-called *meta-states*. These are the length-five vectors of discretized weights characterizing each subject's wFNC at each time window. There are 8^5 possible such meta-states. We investigate several inter-related measures of generic dynamism in this discrete five-dimensional state space: the number of times that subjects *switch* meta-states, the *number of distinct* meta-states occupied, the L^2 -*span* of occupied meta-states, and the overall *distance* traveled in the state space. Schizophrenia was significantly negatively correlated with all four measures of connectivity dynamism. Symptoms with signficant negative effects on all four measures include hallucinations, judgement, social avoidance and mannerism. **Conclusions**: Using a higher-dimensional model of time-varying connectivity we find strong and consistent evidence for reduced connectivity dynamism in SZ patients, with many individual symptoms amplifying this suppression. These distinctive dynamical properties of SZ network connectivity cannot be assessed with conventional static FNC, and were not evident in previous lower-dimensional cluster-based studies of windowed FNCs.

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Poster

760. Network Models and Cognitive Function

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 760.05/VV75

Topic: G.06. Computation, Modeling, and Simulation

Title: Network theory analysis reveals hierarchical cortical organization as a platform for consciousness to emerge

Authors: *N. LAHAV[1], B. KSHERIM[2], E. BEN SIMON[3,4], R. COHEN[2], S. HAVLIN[2];
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Abstract: In recent years scientists have begun to look at the brain as a structural network using graph theory analysis. After several studies revealed that the human brain features small world network characteristics (i.e. small average distance and large clustering coefficient), it is time to address the next step which relates to the possible role of this topology in human brain function. To address this question, we applied k-shell decomposition analysis on a structural human cortical network. In this study, 998 MRI -derived cortical ROIs were used to construct the nodes of each network and 14,865 edges were derived from white matter fibers using DSI. By removing different degrees iteratively, k shell decomposition enables to uncover the most connected area of the network (i.e. the nucleus) as well as the connectivity shells that surround it. These shells correspond to known cortical networks and enable an effective examination of functional cortical organization leading to a comprehensive insight into the hierarchical structure of the human brain. Results reveal that the nucleus was mostly comprised of left hemisphere regions (60%) while right hemisphere regions never reached the nucleus. Furthermore, 70% of the lowest shells were from the right hemisphere. The analysis revealed three major hierarchical groups: the first hierarchy consisted mainly of low shell nodes which are further connected to higher shells. This group contained mostly specified functions such as localized sensory perception and most of its edges were connected to higher hierarchies. The second hierarchy was comprised of high shells, which do not reach the nucleus but share many connections with it. About half of its connections were within the same hierarchy while the other half were connected to the nucleus. The third hierarchical group is the nucleus which formed one component, mostly connected within itself. One can postulate that there is a flow of information from the lowest group to the highest group, with each step enabling further data integration and data processing. The peak of this integration occurs in the nucleus which serves as a global interconnected collective enabling a unified space for global processing. This collective could serve as a platform for consciousness to emerge. In accordance, all of the regions in the nucleus have been previously correlated to conscious activities. This suggestion corresponds with global work space theories and integrated information theory, putting forward the networks nucleus as the perfect candidate for the platform for conscious to emerge

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Poster

760. Network Models and Cognitive Function

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Program#/Poster#: 760.06/VV76

Topic: G.06. Computation, Modeling, and Simulation

Title: Machine intelligence and sensor fusion

Authors: ***H. C. YUAN**[1], M. V. CHAO[2];
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Abstract: The dynamical firing and spiking activity of neurons enables them to communicate. Work conducted by Shandlen and Newsome ("The Variable Discharge of Cortical Neurons", J Neuroscience, 1998) have provided evidence that neurons might integrate information to make decisions. This poster considers decision making models applying sensor fusion in intelligent avionic systems. Avionic systems gather information and data through different sensors as radar and infrared devices. These sensor devices each receive electromagnetic (radio frequency) and optical signals, respectively and digitized. Modern digital processors are programmed in high level languages with algorithms to process data to make real time decisions. The digital processor is the "brain" of these avionic systems, processing data, combining data, making decisions based on the data which are used to control various functions of the system. The algorithms implemented in the processor comprise a "machine intelligence and learning" system. The aim is to supply a combined result or fused summary of the multiple sensors' data for situational awareness. The avionics operator can decide to either command additional sensor data to to conduct a maneuver for tactical reasons or more detail of the situation. The operator of avionics systems can respond rapidly and make other decisions according to the fused information from the sensors. Research in sensor fusion, as illustrated by the research of Ren C. Luo and Chih-Chen Yih of Intelligent Automation Laboratory (IEEE Sensors Journal, Vol. 2, No. 2, pp.107-119, 2002), defines multisensor integration and fusion as the synergistic combination of sensory data from multiple sensors to provide more reliable and accurate information. The potential advantages of multisensor integration and fusion are redundancy, complementarity, timeliness for situational awareness. In avionic systems, machine intelligence and sensor fusion have become more sophisticated and complex with the increases in memory

capacity and speed of modern digital processors, including the application of parallel processing architectures. Avionic systems benefit from the reliability, redundancy, and greater accuracy of the sensor fusion algorithms. Sensor fusion also adds dimension by potentially providing a means to intelligently manage multiple sensors in complex situational awareness scenarios. This poster presents an overview of how Bayesian sensor fusion is applied as machine intelligence. The variability and irregularity of neuronal firing is also considered in the context of simple discrete time models which elaborate on ideas from Markov Chains.

Disclosures: H.C. Yuan: None. **M.V. Chao:** None.

Poster

760. Network Models and Cognitive Function

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Time: Wednesday, November 19, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 760.07/VV77

Topic: G.06. Computation, Modeling, and Simulation

Title: A dynamic functional cartography of cognitive systems

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Abstract: One of the most remarkable features of the human brain is its ability to adapt rapidly and efficiently to external task demands. Novel and non-routine tasks, for example, are implemented faster than structural connections can be formed. The neural underpinnings of such dynamic processing is far from understood. Here we develop and apply novel methods in network science to quantify how patterns of functional connectivity between brain regions reconfigure as human subjects perform 64 different tasks. By applying dynamic community detection algorithms, we identify groups of brain regions that form putative functional communities and uncover changes in these groups across the 64-task battery. We summarize these reconfiguration patterns by quantifying the probability that two brain regions engage in the same network community (or putative functional module) across tasks. These tools enable us to demonstrate that classically defined cognitive systems -- including visual, sensorimotor, auditory, default mode, fronto-parietal, cingulo-opercular and salience systems -- engage dynamically in cohesive network communities across tasks. We define the universal role that a cognitive system plays in these dynamics along the following two dimensions: (i) stability vs. flexibility and (ii) connected vs. isolated. The role of each system is therefore summarized by

how stably that system is recruited over the 64 tasks, or how strongly that system interacts with other systems. Using this cartography, classically defined cognitive systems can be categorized as flexible loners, stable integrators, and anything in between. Our results provide a new conceptual framework for understanding the dynamic integration and recruitment of cognitive systems in enabling behavioral adaptability across a wide variety of tasks. We compare and contrast the theoretical implications of these results with those expected in other models of cognition including global workspace theory.

Disclosures: M.G. Mattar: None. **M.W. Cole:** None. **D.S. Bassett:** None. **S.L. Thompson-Schill:** None.

Poster

760. Network Models and Cognitive Function

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Topic: G.06. Computation, Modeling, and Simulation

Support: Acknowledgments: Funding support from the Department of Anesthesiology and NIH RO1 GM098578-02.

Title: Identification of a general relationship between brain network topology and directed connectivity

Authors: *U. LEE[1,2], J.-Y. MOON[1,2], S. BLAIN-MORAES[1,2], G. A. MASHOUR[1,2,3]; [1]Anesthesiol., Univ. of Michigan Med. Sch., Ann Arbor, MI; [2]Ctr. for Consciousness Sci., Ann Arbor, MI; [3]Neurosci. Grad. Program, Ann Arbor, MI

Abstract: Background: Recent simulation and empirical studies suggest that network topology shapes directed connectivity (sometimes referred to as information flow) in brain networks. However, no general relationship has been systematically studied or identified. **Methods:** In this study we 1) investigated mathematical relationships between network topology and information flow using neural mass models, 2) computationally perturbed brain networks to simulate states of consciousness and unconsciousness, 3) applied graph theoretical network analysis to high-density EEG during consciousness and sevoflurane-induced unconsciousness in humans, and 4) compared results of the model and the experimental data. **Results**: In simple network models, neural mass models and empirical brain networks, hub nodes of higher degree were consistently targets for directed connectivity. After computational perturbation of networks or administration

of sevoflurane in humans, major hub nodes lost degree and directed connectivity was altered accordingly. Furthermore, across neural mass models of rat, cat, monkey, and humans, nodes with the highest degree were consistently "sinks" for information flow. **Conclusion**: A general relationship can describe directed connectivity in brain networks and perturbations of topology predictably alter the direction of information flow. This relationship may have implications for the neurobiology of consciousness across species as well as state transitions in humans.

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